The effect of menstrual phase on fear extinction learning and recall

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A report submitted as a partial requirement for the degree of Bachelor of Psychological Science with Honours in Psychology at the University of Tasmania, 2016

Statement of Sources

I declare that this report is my own original work and that contributions of others have been duly acknowledged.

Cheryl McKay 12 October 2016

Acknowledgments

I would like to thank God for the opportunities He has provided for me in pursuit of a career in psychology. Furthermore, I would like to thank my beautiful partner Chantal for her unwavering support, commitment and encouragement. Without you I would not have gotten this far. You truly are my hero and the wind beneath my wings.

Thank you to my supervisor Professor Kim Felmingham for your dedication, patience and guidance throughout this year. Your ongoing commitment has been greatly appreciated and it has been a pleasure to work with you.

I wish to acknowledge 'the list' and the University of Tasmania for affording me the opportunity to further my education. In addition, my thanks for access to equipment, the psychology research laboratory and students in order to carry out my research.

To my fellow lab rats, thanks for all your support, cake and comradery during this challenging year.

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Word count: 9450

Abstract

Females are twice more likely to develop anxiety disorders as males. One mechanism believed to underlie the development and maintenance of anxiety disorders is impaired fear extinction. Recent studies have considered menstrual phase as a factor that distinguishes males from females in fear extinction recall. The aim of the study was to replicate previous findings and investigate the independent effects of estrogen and progesterone on fear extinction recall. Sixteen males and 29 females (13 in the early-follicular phase, 16 in the mid-luteal phase) were tested in a two-day fear acquisition and extinction task and provided a saliva sample to assess hormonal levels. Skin conductance response was used as the dependent variable in the fear acquisition task. No significant differences were found between mid-luteal females, early follicular females and males during fear acquisition and extinction learning however, early follicular females demonstrated significantly lower fear extinction recall (higher fear recovery) than mid-luteal females. The findings suggest that the mid-luteal phase, with high estrogen and progesterone, may facilitate fear extinction recall in females. This outcome supports the delivery of treatments for some anxiety disorders based on fear extinction, such as exposure therapy, to female clients during the mid-luteal phase of the menstrual cycle.

Fear is a natural response that facilitates safety-seeking behaviour when there is an immediate threat of actual or perceived danger, through arousal of the sympathetic system leading to the activation of the fight or flight response (Dias, Goodman, & Ressler, 2013; VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). The fear response is associated with signs that the body is preparing for action, including tachycardia, tachypnoea, dry mouth, and sweating (Sandin et al., 2015; VanElzakker et al., 2014). These symptoms dissipate rapidly once threat or danger has disappeared, in a process known as fear extinction (Dias et al.). While symptoms of anxiety mirror those of fear, anxiety disorders are characterised by symptoms arising in the presence of non-threatening stimuli (Dias et al.; Graham & Milad, 2011). In addition, unlike a natural fear response, pathological anxiety reflects a persistent and disabling increase in arousal and hypervigilance toward both external stimuli and internal sensations which leads to maladaptive behaviours such as avoidance (Dias et al.; Duits et al., 2015). Sandin et al. argue that it is the cognitive appraisal of events, behaviours, and bodily sensations that becomes distorted and accentuates the catastrophic thinking typical of anxiety disorders. Moreover, VanElzakker et al. suggest that malfunction in fear extinction may be an underlying factor leading to the onset of anxiety disorders.

Epidemiological studies consistently reveal that females are twice as likely to develop anxiety disorders compared to males (e.g., Kessler et al., 2005). Furthermore, a recent meta-analysis conducted by Steel et al. (2014) found the global lifetime prevalence of anxiety disorders was higher for females than males. Importantly, although the prevalence of anxiety disorders is lower in non-western cultures, gender effects remain prominent cross-culturally (Steel et al.). Given that males have a higher frequency of exposure to fear-evoking events (e.g., war,

violence) than females it appears counter-intuitive that females render higher prevalence rates of anxiety disorders (Breslau et al., 1998). Nevertheless, females report higher anxiety symptom severity and disability (Lonsdorf et al., 2015). Kelly and Forsyth (2007) propose biological effects may exist that inform the observed sex differences. Taking into account the view that impaired fear extinction may underlie the development and maintenance of anxiety disorders, this study aims to investigate the effect of menstrual phase (with varying levels of estrogen and progesterone) on fear acquisition, extinction learning, and extinction recall in males and females.

Fear Acquisition and Extinction

A simple yet effective way of studying anxiety related psychopathology is to use a basic fear acquisition and extinction task (Lonsdorf et al., 2015), as illustrated in Figure 1. Initially the two neutral stimuli (here, pink and blue crosses) are presented on their own (habituation). During acquisition one of the two neutral stimuli is paired with an unpleasant, unconditioned stimulus (US) such as an electrical stimulus (Stockhorst & Antov, 2016). After numerous pairings of the neutral stimulus with the US, the neutral stimulus becomes a conditioned stimulus (CS^+) and a fear response becomes a conditioned response (CR) to the CS^+ (Kelly & Forsyth, 2007; Stockhorst & Antov). The other neutral stimulus, referred to as the CS^-, denotes a safety signal as it is never paired with the electrical stimulus (Duits et al., 2015; Stockhorst & Antov). Subsequently, the presentation of the CS^+ elicits the CR in the absence of the US, due to heightened expectancy of the aversive stimulus and fear associated with the CS^+ (Stockhorst & Antov). In the early and late fear extinction phases, the CS^+ and CS^- are presented multiple times without the US, leading to a diminished CR in response to the CS^+ (Zorawski et al., 2005). Then, as shown in Figure 2, extinction learning recall is tested approximately 24 hours later.

This encompasses measuring the memory of fear extinction learning, which occurred the day before by presenting multiple trials of the CS^+ and CS^- stimuli without the aversive stimulus (Graham & Milad, 2011).

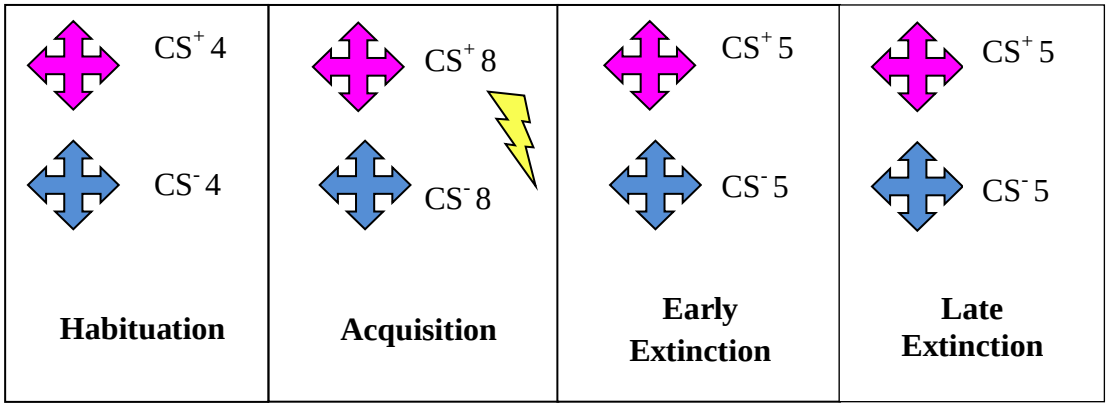

Figure 1. A typical fear acquisition and extinction paradigm with CS^+ and CS^- trials on day one.

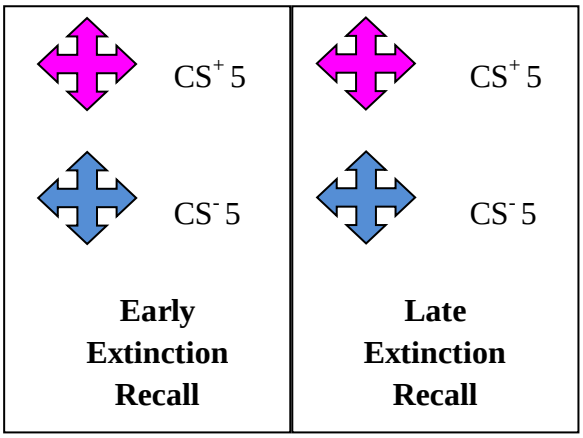

Figure 2. Fear extinction recall phase on day two of a fear acquisition and extinction paradigm with CS^+ and CS^- trials.

To determine whether fear acquisition and extinction have taken place, the skin conductance response (SCR) has been used as an objective measure of sympathetic arousal to the presentation of CS^+ and CS^- stimuli. SCR is an indicator of increased electrical connectivity that occurs in the skin as a consequence of physiological arousal (Critchley, Elliot, Mathias, & Dolan, 2000). Higher SCR indicates higher sympathetic response (e.g. fear response) and lower SCR indicates

lower sympathetic response (e.g. no fear response) (Kelly & Forsyth, 2007; Bach & Friston, 2013). That is, SCR offers an objective, physiological insight into an individual's subjective response to the feared stimulus (Vansteenwegen et al., 2005). As illustrated in Figure 3, SCR amplitude (indexed as baseline in habituation) rises shortly after fear acquisition begins. Typically, with numerous pairings of the CS^+ with an aversive stimulus, SCR with the CS^+ escalates and remains higher than that of the CS^-, even in the absence of the US. High SCR in CS^+ trials indicates the expectation of danger and lower SCR in CS- trials indicates the identification of safety (White & Graham, 2016). Extinction learning and extinction recall is marked by SCR amplitude returning to near-baseline levels (Vansteenwegen et al.; Otto, Moshier, & Kinner, 2014). SCR amplitude that remains significantly higher than baseline is indicative of poor fear extinction and poor extinction recall (Vansteenwegen et al.).

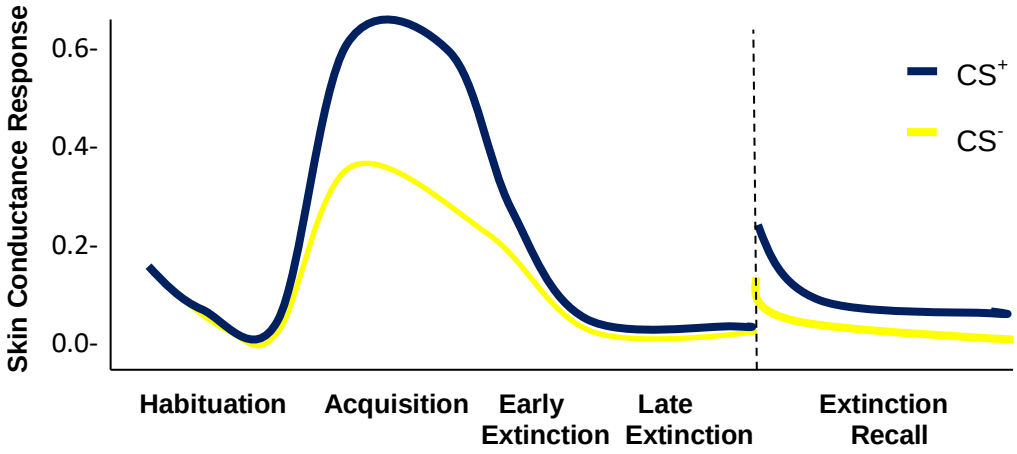

Figure 3: An example of SCR amplitude to the CS^+ and CS^- in a two–day fear conditioning and extinction paradigm (adapted from Homberg, 2012).

While respecting ethical constraints to inducing fear acquisition (such as the imperative to reduce potential harm), conducting fear acquisition and extinction tasks within a laboratory setting enables researchers to closely examine the processes of fear acquisition and fear extinction (Jackson, Payne, Nadel, & Jacobs, 2005;

Wegerer, Kerschbaum, Blechert, & Wilhelm, 2014). Furthermore, whilst using a fear acquisition paradigm does not replicate the exact conditions under which fear acquisition takes place in the 'real world', eliciting a fear response adds emotional properties to the conditioned stimulus and thus provokes 'real life' symptoms (Miskovic & Keil, 2012). In addition, the paradigm affords the examination of associated learning and development of threat expectancy as a consequence of that associated learning (Duits et al., 2015). Obtaining SCR and subjective threat-expectancy ratings from participants, allows direct observation of normal and dysfunctional fear extinction as well as extinction memory within a controlled environment (Graham & Milad, 2011; Miskovic & Keil; Wegerer et al.; Zorawski et al., 2005).

Sex Differences in Fear Acquisition and Extinction

Early investigations of sex differences in fear extinction and recall yielded inconsistent findings. Some studies found that males demonstrated greater fear acquisition (higher SCR) versus females while other studies found the direct opposite (Guimareas, Hellerwell, Hensman,Wang, & Deakin, 1991; Inslicht et al., 2013; Milad et al., 2010; Zorawski et al., 2005). In addition to varying fear acquisition effects, there have been inconsistent findings in extinction recall between males and females (Lonsdorf et al, 2015; Milad et al., 2006). For example, Shevil et al. (2014) studied fear acquisition and recall in trauma exposed and PTSD-diagnosed males and females. Conducting a fear acquisition and extinction task the authors found males had greater fear extinction recall compared to females. In contrast, studying healthy male and female participants, Milad et al. (2006) found that females exhibited greater fear extinction recall compared to males. Dissimilarly, Lebron-Milad et al. (2012), who also studied healthy males and females, found no significant differences in fear

extinction recall between males and females. The variability in research outcomes in both clinical and healthy participants has consistently pointed to one potential explanation for these inconsistent findings as a failure to control for menstrual phase in females (Milad & Maeng, 2015; Shevil et al.).

The Human Menstrual Cycle

The human menstrual phase is divided into two phases, based on estrogen and progesterone hormone levels, with the average menstrual cycle typically lasting 28 days (Figure 4; Nillini, Toufexis & Rohan, 2011). The follicular (early and late) phase occurs between days one and 14 of the menstrual cycle (Wilson, Carvalho, Granot, & Landau, 2013). During the early follicular (days 1-7), estrogen and progesterone levels are both low, whereas in the late follicular (days 7 to 14) progesterone levels remain low while estrogen levels rise and peak (Graham & Milad, 2013). In the luteal phase (days 16 - 28), while estrogen remains higher than in the early follicular phase, it is dominated by elevated progesterone (Wilson et al.). During days 18 to 24, termed the mid-luteal phase, there is a peak in progesterone levels followed by the late luteal phase (days 24-28) when both estrogen and progesterone decline (Wilson et al., 2013). Females tend to report notably more symptoms of anxiety during episodes of low estrogen (pre-menstruation, menstruation, menopause, and postpartum; Glover et al., 2013). According to Graham and Milad, differences in fear extinction recall observed between males and females may arise from fluctuating sex hormones. Furthermore, the divergence of estrogen seen in females over the menstrual cycle may offer an explanation for the observation of varying extinction recall in females (Milad et al. 2006).

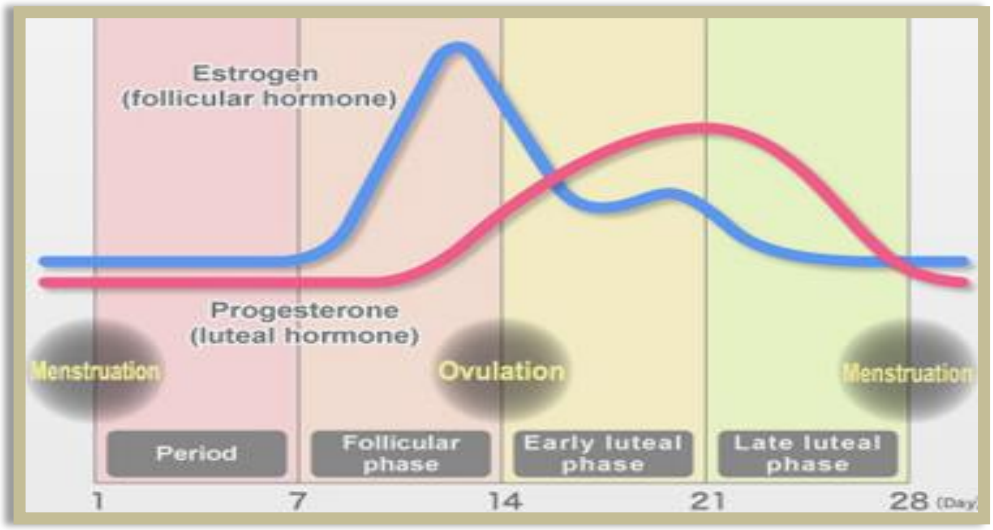

Figure 4: Varying estrogen and progesterone levels across the follicular and luteal phases in the human menstrual cycle (taken from Otsuka Pharmaceuticals, 2016).

The Impact of Menstrual Phase on Fear Acquisition, Extinction Learning and Extinction Recall

Milad et al. (2010) studied fear extinction and extinction recall in 18 healthy males and 36 healthy females not using hormonal contraception. A blood sample from each participant was drawn for rigorous measurement of estrogen and progesterone levels, in order to accurately assess sex hormone influence on fear acquisition, extinction learning, and extinction recall (Jackson et al., 2005). Based on the pathology results, females were subsequently separated into two groups, early follicular (18 cases) and late follicular (18 cases). All participants underwent a two-day fear acquisition and extinction paradigm. Coloured lights were used as the CS^+ and CS^-, an electrical stimulus was the US, and SCR was the measured throughout the paradigm. In addition, to assess extinction recall (memory of fear extinction) on day two, a fear retention index (a measure of the recovered fear response to a CS^+ presented post-extinction learning) was calculated for each participant. The largest CS^+ SCR during conditioning was divided by the mean SCR of the first two trials

during extinction recall, and multiplied by 100 (Milad et al.). Overall, no significant differences were found in fear acquisition or extinction between the early follicular and late follicular females. When the data on females were pooled, males had significantly higher SCR during fear acquisition compared to females, suggesting males experienced greater conditioning effects. Nevertheless, there was no significant difference between males and females in the fear retention index (Milad et al). However, when the fear retention index was analysed by group (males, early follicular females, and late follicular females), the early follicular females demonstrated significantly higher fear retention, signifying greater fear response to CS^+ stimuli, compared to late follicular females and males. While these results imply that estrogen facilitates fear extinction recall (Milad et al), the study did not include females in the mid-luteal phase, which limits the assessment of extinction recall across menstrual phase.

Extending this research, Graham and Milad (2013) conducted a study predicting that females with high estrogen levels would exhibit better fear extinction recall compared to females with low estrogen levels. Participants were 13 females on chemical contraception and 32 naturally-cycling females (blood serum results determined 16 low estrogen females; 16 high estrogen females) who completed a two-day fear acquisition and extinction task. Here, the CS^+ constituted pictures of lamps paired with an electrical stimulus. On day one, there were no significant differences in SCRs in acquisition and extinction learning across groups (Graham & Milad). On day two, a fear retention index (measuring fear recovery in extinction recall) was calculated by taking the mean SCR for all extinction recall trials and dividing it by the highest CS^+ SCR in the conditioning phase, and multiplying by 100 (higher percentage indicates poorer extinction recall; Graham & Milad; Milad et al,

2010). Analysis found that females with low estrogen and females on chemical contraception (chronically low estrogen) had higher fear retention indices, reflecting impaired fear extinction recall, compared to females with high estrogen. This suggests that low estrogen levels impair extinction learning recall (Milad & Graham). Furthermore, accumulating evidence suggests that the activation of a fear response enables rapid adaptation to the presence of threat (experimental acquisition) and the rapid extinction of fear responses (decreased SCR to CS^+ stimuli within session) during early and late experimental extinction, facilitates fear extinction learning (Stockhorst & Antov, 2016). However, as poor extinction recall is indexed by an elevation in fear recovery when original threat-conditioned stimuli (CS^+ and CS^- stimuli without the aversive stimulus) are seen again (usually 24 hours later), it is the failure of extinction learning memory that may lead to psychopathological symptoms of anxiety (Lonsdorf et al., 2015).

Following on from the above study, Graham and Milad (2013) further explored estrogen as an important factor in fear extinction in 31 naturally-cycling females with low estrogen levels. The fear conditioning task differed from the typical paradigm, being conducted over three days to assess recall over a longer period than the traditional 24 hours. Day one comprised fear acquisition, day two covered fear extinction training, and extinction recall was assessed on day three. On day one, participants completed the acquisition tasks. On day two, the participants were randomly assigned to a placebo or oral estradiol administered group prior to completing the extinction training. Analysis of SCR data found no significant differences in fear extinction between the two groups. On day three, the fear retention index was calculated. Analysis revealed that females receiving placebo demonstrated significantly higher scores on the fear retention index (calculations

explained above) than the estrogen-treated group (Graham & Milad). The authors concluded that estrogen facilitates the consolidation of extinction memory, in line with previous evidence (Graham & Milad). Moreover, with a wealth of evidence supporting progesterone in memory consolidation it is surprising that the possible effects of progesterone weren't explored in this study (Maeng & Milad, 2015).

Wegerer et al. (2014) addressed the roles of estrogen and progesterone in fear acquisition and extinction, and intrusive memories, with an emphasis on the effects of hormonal levels over menstrual phase. Naturally-cycling healthy females were recruited. Eighteen females in the early follicular phase were tested between days one and seven of their menstrual cycle, and 21 females in the luteal phase were tested approximately five days before menstruation was due to commence. Before commencing the experiment, participants provided a saliva sample to measure hormonal levels. The fear acquisition and extinction task exposed participants to either an auditory stimulus paired with a violent video as the CS^{+} or an auditory stimulus alone as CS^{-}. In addition, participants completed an online intrusive-memory questionnaire once they had left the laboratory and for two days thereafter (prior to going to bed). Differences in SCR were obtained by subtracting the SCR in CS^{-} trials from the SCR in CS^{+} trials. ANOVAs found no significant differences between the early follicular and luteal females in either habituation or fear acquisition, but significantly higher differential SCR was noted in the early follicular females during the extinction phase (directly after acquisition) compared to luteal females. Correlation analysis found early follicular females experienced significantly higher intrusive memory strength (evaluated by distress, frequency, and duration of memories) than luteal females. The study's conclusions were that estrogen facilitates extinction learning and that low estrogen may elevate the prevalence of intrusive

memories. It is interesting that, despite the authors' emphasis on determining hormonal influences on fear-related responses (SCR), they did not include an additional day to further examine how estrogen and progesterone affect extinction recall. Moreover, the study included females in the late luteal stage when both progesterone and estrogen decline, i.e. progesterone is not at its peak, meaning that fear extinction effects between estrogen and progesterone could not be accurately discriminated (Nillini, Toufexis & Rohan, 2011).

Contributing to this line of research, White and Graham (2016) recently studied fear acquisition, extinction, and extinction recall, as well as the relationship between threat expectancy and SCR response. Participants were males, females using contraception (chronically low in estrogen), and naturally-cycling females. All participants completed a two-day fear acquisition and extinction task, whereby the CS^+ was paired with an electrical stimulus during the acquisition phase (day one). On day two, the fear retention index was calculated as per Milad and Graham (2013). Blood serum analyses of estrogen and progesterone saw naturally-cycling females allocated to a high-estrogen high-progesterone or low-estrogen-low progesterone group. A regression analysis rendered estrogen as a significant predictor of extinction recall, with no significant variance explained by progesterone. Moreover, progesterone levels were not significantly different between the low estrogen, high estrogen, and contraception female groups. Consequently, White and Graham analysed all data based on high or low estrogen levels only. No significant SCR variations were seen between the four groups in habituation, acquisition and early/late extinction learning. On day two, fear recovery was significantly higher in contraception users than in high-estrogen females. Interestingly, unlike previous research that has found naturally cycling low estrogen females depict significantly

poor extinction recall compared to high estrogen females (Graham &Milad, 2013; Milad et al., 2010), no significant difference in fear recovery was found between males, females with low estrogen or females with high estrogen. The authors concluded that chronically low estrogen levels hinder fear extinction recall. These findings correspond with those of Graham and Milad, who found diminished fear extinction recall in females on contraception. Even though White and Graham controlled for the influences of sex hormones, the failure to specifically target mid-luteal females as progesterone peaked may have added additional insight into the effects of divergence of progesterone across the menstrual phase. Nevertheless, the non-significant differences in progesterone levels foreshadowed the nonsignificant finding of progesterone as a predictor of fear extinction recall.

The Effect of Progesterone on Fear Acquisition, Extinction Learning and Extinction Recall

The literature reviewed above demonstrates strong support for estrogen as an important mediator in fear extinction recall in females. Interestingly, while researchers have emphasised the importance of menstrual phase, progesterone (known for its role in memory consolidation; Felmingham, Fong, & Bryant, 2012) has, for the most part been, neglected. To our knowledge, only one human study has directly considered peak progesterone in mid-luteal females in a two-day fear acquisition and extinction task using a within-subjects design. Pineles et al. (2016) examined trauma-exposed females with and without posttraumatic stress disorder (PTSD) in both the mid-luteal (high estrogen, high progesterone) and early follicular (low estrogen, low progesterone) phase. Due to known effects of poor progesterone synthesis in females with PTSD (Rasmusson et al., 2006), Pineles et al. predicted that extinction recall would be comparable in PTSD females over both the early follicular

and mid-luteal phases. Blood samples confirmed hormone levels and menstrual phase. Participants completed the two-day fear acquisition and extinction task twice: once in the early follicular phase and again in the mid-luteal phase. Trauma exposed females were found to have poorer extinction recall in the early follicular phase compared to the mid-luteal phase, which replicates findings by Milad and Graham (2013). However, females with PTSD showed the opposite effect, with poorer extinction recall in the mid-luteal phase compared to the early follicular. Females with PTSD may experience variable neurological effects of sex hormones compared to healthy females (not reviewed in this thesis), which may explain the unexpected impaired fear extinction when estrogen and progesterone were high. However, improved extinction recall demonstrated by the trauma exposed females in the mid-luteal phase suggests a possible role for progesterone when combined with high estrogen in fear extinction recall.

Pineles et al. (2016) study was limited by using a within-subjects design; women completed the fear acquisition and extinction task at two stages of their menstrual phase. Whilst a within-subjects design has the advantage of reducing individual differences, completing the conditioning task twice practice effects may have influenced results. Furthermore, the lack of a healthy control group prevents comparison between clinical and healthy populations. Since this is the only human study that has specifically examined mid-luteal females in relation to fear extinction, further research is required.

In summary, recent research suggests sex hormones (specifically estrogen) have a significant role in fear extinction and recall (Graham & Milad, 2011). While results appear consistent with no significant differences in fear acquisition between males and females in a single session, the impact of sex hormones becomes evident

when assessing the recall of fear extinction using a two-day paradigm (Milad et al., 2010). Understanding the impact that menstrual cycle and levels of estrogen and progesterone have on fear conditioning, extinction learning and recall in healthy humans may offer a deeper understanding into the sex differences that have been observed in the prevalence of anxiety disorders (Lonsdorf et al., 2010).

Therefore, the aims of the current study are, firstly, to replicate previous findings that females with low estrogen have poor fear extinction recall in a two-day fear acquisition and extinction paradigm, and, secondly, to investigate the independent effects of estrogen and progesterone on fear extinction recall in healthy females and males. The first hypothesis is that healthy females in the early follicular (low estrogen) phase will demonstrate significantly poorer fear extinction recall, indexed by greater SCR to a conditioned stimulus on day two of the experiment, compared to healthy males and healthy females in the mid-luteal phase. The second hypothesis, analysing the independent effects of estrogen and progesterone, predicts that a negative relationship between estrogen and SCR amplitude on day 2 (reflecting impaired extinction recall with lower estrogen) will be present but there is no specific directional relationship relating to progesterone given the dearth of current literature.

Method

Participants

According to a power analysis a sample size of 60 participants was required. Participants were recruited from the first-year psychology cohort at the University of Tasmania (UTAS), who were awarded 1.5 hours of course credit, and other students across the campus who responded to advertisements were reimbursed $20 for their time. The current study was built on a database previously collected at UTAS (To, 2015). The existing database comprised 12 males and 15 females in the early

follicular phase. Due to difficulty in recruiting naturally cycling females, seven of the female participants were on oral contraception and were tested during the sugar-pill week (days one to seven of the menstrual cycle). According to Pluchino et al. (2009), estrogen levels during menstruation of females using oral contraception are similar to naturally-cycling females, justifying their inclusion in this study. The current study extended the database by recruiting an additional six males, one female in the early follicular phase and 17 females in the mid-luteal phase. Data from two males, one mid-luteal female, and three early follicular females were excluded, two for technical reasons, three for abnormal hormonal levels and one voluntary withdrawal. Following the exclusions, a total of 45 participants: 16 males with mean age of 25.44 years (*SD* = 5.84) and 29 females with mean age of 24.03 years (*SD* = 6.80) completed the study. The female participants formed two groups: early follicular comprised 13 individuals (7 naturally-cycling, 6 on oral contraception) and a mid-luteal (naturally-cycling) group comprised 16 individuals. The current study excluded any females over the age of 45 years to control for effects on hormone levels of menopause, irregular menstrual cycles, and any form of hormonal contraception (oral, nuvaring, etonogestrel implant, hormone covered intrauterine devices and depot medroxyprogesterone acetate injections) and any other hormonal medication were excluded . Individuals with cardiovascular, neurological and psychiatric disorders, and anyone on steroids were also excluded. Participants were briefly interviewed about medication usage and medical history prior to commencing the experiment. Participants disclosing any exclusion criteria during the interview were not included in the study.

Materials and Measures

Ethics approval (Appendix A1) was obtained from the Social Sciences Human Research Ethics Committee at UTAS. An information sheet (Appendix A2) was sent to each participant prior to the experiment.

Medical checklist. A medical and medication use checklist was used to record current medication use, prior and current medical history of neurological, psychological and cardiovascular disorders (Appendix B1). Clinical and demographic information was also recorded on the checklist.

The Depression Anxiety Stress Scale (DASS 21). The DASS 21 (Cronbach's alphas of .94 for depression, .87 for anxiety and .91 for stress) was completed to gain insight into participants' anxiety, stress and depressive state over the last week (Appendix B2). Participants responded to statements such as, "I felt that I had nothing to look forward to" on a four point Likert scale (Lovibond & Lovibond, 1995). Scores were multiplied by two to gain a score out of 42.

The Difficulty Emotion Regulation Scale (DERS). The DERS (with a Cronbach's alpha of 0.93) was completed to assess the participants' emotional regulation and emotional awareness at the start of the experiment. Participants rated their responses to statements for example, "I experience my feelings as overwhelming and out of control" on a 5 point Likert scale (AppendixB3; Gratz & Roemer, 2004).

The Catastrophic Cognitions Questionnaire-Modified (CCQ-M). The CCQ-M (Cronbach's alpha of 0.88) was administered to establish an individuals' perception of how dangerous they find mental and bodily sensations. Responses were made on a 5 point Likert scale to phrases such as "unable to control thinking" (Appendix B4; Khawaja, Oei, & Baglioni, 1994).

The Beliefs about Emotions Scale (BAES). The BAES (Cronbach's alpha of 0.91) was used to ascertain attitudes towards experiencing and revealing negative emotions to others for example, "It would be a sign of weakness to show my emotions in public". Responses were made using a 6 point Likert scale (Appendix B5; Rimes & Chalder, 2010).

Saliva samples. Saliva samples were collected using the passive drool method (Gallagher, Leitch, Massey, McAllister-Williams, & Young, 2006) in saliva collection tubes, and assayed using standardised Salimetrics salivary Elisa and progesterone assay kits at a pathology laboratory at Macquarie University to assess estradiol (E_2, the dominant form of free bioavailable estrogen) and progesterone levels, and to confirm menstrual phase. Thawed salivary samples were centrifuged at 1500 x *g* for 15 min.

Fear acquisition and extinction. A two-day fear acquisition and extinction paradigm (adapted from Milad et al., 2006) was used for the experiment. Skin conductance electrodes, AD Instruments, Inquisit 3.0.6.0 program and lab chart software were used to conduct the fear conditioning and extinction task and record output data. A Powerlab 16/35 Stimulus Isolator was used to deliver the electrical stimulus.

Procedure

Female participants contacted the researcher either by telephone or email on the first day of menstruation. Females allocated to the early follicular group were booked into the Psychology Research Centre to complete the experiment between days two and seven of their menstrual cycle. Females allocated to the mid-luteal group completed the experiment on days 19 and 20 or 20 and 21of their cycle. On day one, the procedure was explained and written informed consent obtained

(Appendix A3). A checklist confirming medical history, hormonal contraception and medication use was completed. Participants completed the DASS 21, DERS, CCQ-M, and BAES questionnaires. A saliva sample was collected, labelled and frozen at-20°C. SCR electrodes were placed on the first and third finger of the non-dominant hand, and the electrical stimulus electrode placed between the thumb and index finger on the dominant hand. Participants chose the strength of the electrical impulse starting at 2mA and increasing by 0.5mA, until the stimulus was reported to be significantly uncomfortable but not painful. The system was calibrated to zero (before establishing baseline SCR for each participant) prior to commencing the fear acquisition and extinction paradigm. Day one of the fear acquisition and extinction paradigm was then completed. The visual stimuli consisted of orange and green circles. The CS^+ function was randomly assigned in order to counterbalance the stimuli among participants.

The green and orange circles, 7 centimetres in diameter, were presented on a screen for twelve seconds. The inter-trial intervals ranged between 12 and 21 seconds with a mean of 16 seconds. Prior to commencing the experiment, participants were asked to keep as still as possible, in order to reduce SCR artefact. As shown in Figure 5, day one comprised of four phases: habituation, acquisition, early extinction, and late extinction. For the habituation phase, participants were advised that they would not receive an electrical stimulus. The habituation phase presented eight trials (4 green circles, 4 orange circles) in a randomised order. This was followed by the acquisition phase. Prior to the commencement of the acquisition phase participants were informed they may or may not receive an electrical stimulus and were reminded to keep as still as possible. The acquisition phase presented a total of 16 trials (8 green circles, 8 orange circles). Five out of the eight CS^+ trials were paired with a 50-

millisecond electrical stimulus (62.5% reinforcement). Acquisition was followed by the early extinction phase, which presented 10 trials (5 green and 5 orange circles). Finally, the late extinction phase presented 10 trials (5 green and 5 orange circles).

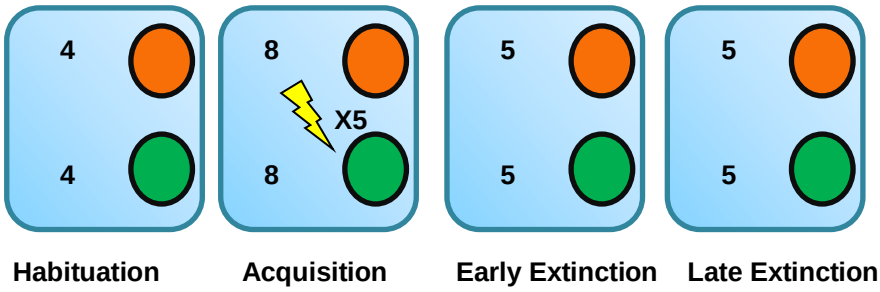

Figure 5: Phases and trials of the fear acquisition and extinction paradigm on day one.

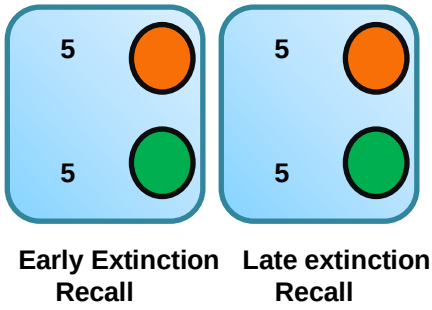

Figure 6: Phases and trials of fear extinction recall on day two.

Participants returned to the laboratory the following day (day two), for the assessment of extinction recall. The SCR electrodes and electrical stimulus electrode were attached and the system calibrated as per day one. Participants were reminded to keep as still as possible during the task. No instructions regarding the presence or absence of the electrical stimulus were given. As illustrated in Figure 6, the early extinction recall and late extinction recall phases replicated the extinction phases on day one, with 10 trials (5 green and 5 orange circles) in early extinction recall and 10 trials (5 green and 5 orange circles) in late extinction recall. In addition, for contingency awareness of the CS^+ and CS^-, as a manipulation check, participants

were asked if they knew which colour was associated with the electrical stimulus. Participants were then debriefed and thanked for their participation.

Design and Analysis

Consistent with previous research, a repeated measure between-factors design was used, with repeated measures mixed-model analyses of variance (ANOVA) to analyse data (Glover et al., 2012; Inslicht et al., 2013; Wegerer et al., 2014). The average SCR of the 2 second pre-stimulus mean was calculated to gain a baseline skin conductance level for each participant. To measure the change in SCR amplitude the two-second pre-stimulus mean was subtracted from the highest SCR value recorded during the 12-second CS presentation for each trial in each phase. In line with previous research, each SCR value was square root transformed to reduce heteroscedasticity. For negative values, the absolute number was found, square root transformed, and the negative sign replaced (Inslicht et al., 2013; Milad et al., 2010). Data collected by To (2015) and data from the current study were collated and analysed using SPSS version 23.

To test hypothesis one, a series of separate 3 (Group: males, early follicular females, mid-luteal females) x 2 (Condition: CS^+, CS^-) x 4 or 5 (Trial) repeated measures ANOVAs were conducted, with SCR amplitude as the dependent variable. Trial one in the acquisition phase was not included in the analysis, as an increase in SCR in the first trial is probably a consequence of given instructions (may or may not receive an electrical stimulus) rather than conditioning (Inslicht et al. 2013) as no electrical stimulus pairings had been made. Trials after trial five in acquisition were not analysed as SCR tends to decline towards the end of the acquisition phase (Milad et al., 2010). To analyse extinction recall, the percentage value of the fear retention index was calculated by dividing the combined average SCR for early extinction

recall and late extinction recall trials by the maximal SCR for a CS^+ during fear acquisition and multiplying by 100. This was followed by a one-way ANOVA to test differences across groups (Graham & Milad, 2011; Milad et al., 2010). For one-way ANOVA's Levene's test of homogeneity of variance was checked for violation (Allen & Bennett. 2012). For repeated measure ANOVAs, Greenhouse-Geisser correction was applied when Mauchly's test of sphericity was violated (Field, 2009). For multiple comparisons, Sidak corrections were used with the alpha level set at .05 for statistical significance (Allen & Bennett. 2012). To test hypothesis two, a hierarchical regression was conducted with fear recovery index as the outcome variable and estradiol and progesterone as the predictor variables (White & Graham, 2016). Cooks distance was used to determine possible outliers and the VIF and Tolerance were checked for multicollinearity (Allen & Bennett, 2012).

Results

Demographic and Clinical Data.

Univariate ANOVAs were conducted on demographic and clinical data, with means and standard deviations presented in Table 1. There were no significant differences between the groups in scores on the DASS (Depression, Anxiety, and Stress), CCQ-M, DERS, BMI, hours awake, or stimulus intensity. Salivary levels of estradiol, $F(2, 42) = 3.54$, $p = .038$, $\eta_p^2 = .14$, and progesterone, $F(2, 42) = 14.76$, $p < .001$, $\eta_p^2 = .41$, were significantly different between the groups (group means are included in Table 1 and see Figure 7). Assays with a coefficient of variation (CV%; repeated measurement of same sample to determine deviation and consistency) <10% were considered for analysis (Chesher, 2008; Salimetrics Inc; Appendix C).

Table 1.

Demographic and Clinical Data for Early Follicular Females, Mid-luteal Females and Males

	Early Follicular Females	Mid-luteal Females	Males	*F*	*p*	η_p^2
	M SD	*M* SD	*M* SD			
DASS Depression	2.92 (2.14)	4.38 (4.46)	3.81 (4.89)	0.45	.641	.02
DASS Anxiety	5.00 (4.81)	4.38 (4.46)	3.88 (6.43)	0.16	.849	.01
DASS Stress	6.62 (3.80)	8.69 (5.87)	6.13 (6.76)	0.89	.418	.04
CCQ-M	58.38 (13.79)	60.56 (13.50)	50.19 (14.69)	2.41	.103	.10
DERS	90.69 (20.67)	75.00 (16.36)	82.34 (16.64)	2.70	.073	.12
Age	25.00 (7.48)	23.25 (6.32)	25.44 (5.84)	0.50	.611	.02
BMI	21.52 (3.18)	23.08 (4.40)	23.25 (3.75)	0.85	.436	.04
Hours awake	6.65 (2.19)	5.03 (3.11)	5.63 (2.99)	1.19	.314	.05
Stimulus (mA)	1.8 (0.5)	2.1 (0.5)	2.1 (0.3)	1.76	.185	.07
Progesterone	38.46 (49.50)	161.11 (111.55)*	34.61 (28.79)			
Estradiol	2.07 (0.63)	2.57 (0.65)	1.95 (0.77)*			
CS^+ ID (*N*)	8	10	13			

Note. CS^+ ID = CS^+ correct identification. * $p < .05$

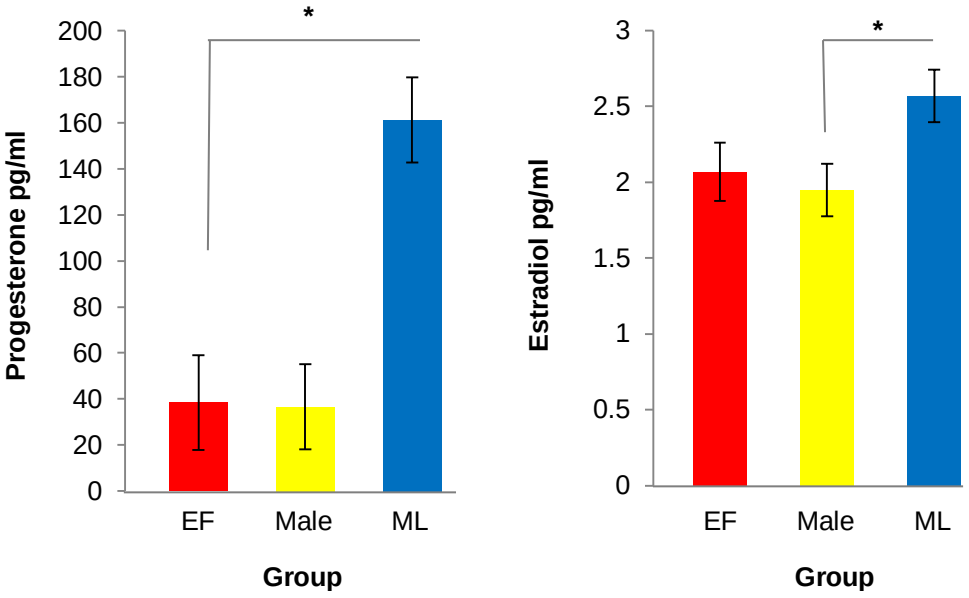

Figure 7. Mean progesterone and estradiol salivary levels in the early follicular, male and mid-luteal groups. Error bars are standard error. * p <.050.

For progesterone the assay sensitivity was 5.0 pg/ml. The intra-assay variability was 5.4% and inter-assay variability was 5.9%. For estradiol levels the assay sensitivity was 0.1 pg/ml. The intra-assay variability was 5.9% and the inter-assay variability was 6.4%. Since a one-way ANOVA found no significant differences in baseline SCR across groups, $F(2, 42) = 0.08$, $p = .924$, $\eta_p^2 = .004$, group differences found between early follicular females (EF), mid-luteal females (ML) and males are not attributed to the findings presented below.

Skin Conductance Response

Data were screened for missing values and outliers. No data points were missing. Two data points (one in the ML group and one in the male group) were identified as outliers. These two values were corrected to three standard deviations above the mean before analyses were conducted (Tabachnick & Fidell, 2001). Mean SCR for each phase on day one is presented in Figure 8.

Habituation. A 3 x 2 x 4 (Group [male, EF, ML] x Condition [CS^+, CS^-] x Trial [1, 2, 3, 4]) repeated measures ANOVA revealed a significant effect of trial,

$F(3, 126) = 9.90$, $p < .001$, $\eta_p^2 = .19$. Sidak-corrected pairwise comparisons found SCR in trial 1 ($M = 0.76$) was significantly higher than trial 3 ($M = 0.47$), $p < .001$, 95% CI [0.12, 0.47] and trial 4 ($M = 0.53$), $p = .011$, 95% CI [0.04, 0.42]. In addition, SCR in trial 2 ($M = 0.72$) was significantly higher than trial 3, $p = .004$, 95% CI [0.06, 0.44] and trial 4, $p = .045$, 95% CI [0.01, 0.37]. No significant effect of group, $F(2, 42) = 0.30$, $p = .746$, $\eta_p^2 = .01$, or condition, $F(1, 42) = 0.10$, $p = .756$, $\eta_p^2 = .002$, was found. There was no significant interaction for Condition x Group $F(2, 42) = 1.66$, $p = .200$, $\eta_p^2 = .07$, Trial x Group, $F(6, 126) = .81$, $p = .567$, $\eta_p^2 = .04$, Condition x Trial, $F(3, 126) = 0.31$, $p = .815$, $\eta_p^2 = .01$, or for Group x Condition x Trial, $F(6, 126) = 0.72$, $p = .637$, $\eta_p^2 = .03$.

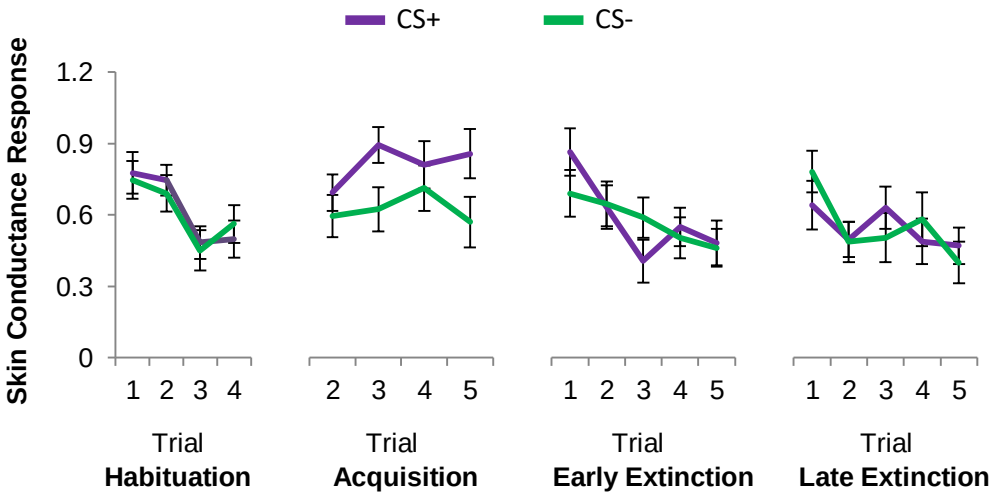

Figure 8. Mean skin conductance, collapsed across groups, in habituation, acquisition, early extinction and late extinction. Error bars are standard error.

Acquisition. A 3 x 2 x 4 (Group [male, EF, ML] x Condition [CS^+, CS^-] x Trial [2, 3, 4, 5]) repeated measures ANOVA found a significant effect of condition, $F(1, 42) = 9.15$, $p = .004$, $\eta_p^2 = .18$. A Sidak-corrected pairwise comparison found that SCR in the CS^+ condition was significantly higher ($M = 0.81$) than the CS^- condition ($M = 0.63$), $p = .004$, 95% CI [0.06, 0.31], implying SCR increased during fear conditioning. There were no significant effects of group, $F(2, 42) = 0.002$, $p =$

.998, $\eta_p^2 < .00$, or trial, $F(3, 126) = 0.87$, $p = .458$, $\eta_p^2 = .02$. There was no significant interaction between Condition x Group, $F(2, 42) = 1.23$, $p = .303$, $\eta_p^2 = .05$, Trial x Group, $F(6, 126) = 0.47$, $p = .830$, $\eta_p^2 = .02$, Condition x Trial, $F(3, 126) = 1.15$, $p = .332$, $\eta_p^2 = .03$, or Group x Condition x Trial, $F(6, 126) = 0.97$, $p = .455$, $\eta_p^2 = .04$.

Early extinction. A 3 x 2 x 4 (Group [male, EF, ML] x Condition [CS^+, CS^-] x Trial [1, 2, 3, 4, 5]) repeated measures ANOVA established a significant effect of trial, $F(3, 129) = 5.51$, $p = .001$, $\eta_p^2 = .12$. Sidak-corrected pairwise comparisons found SCR amplitude for trial 1 ($M = 0.78$) was significantly higher than trial 3 ($M = 0.50$), $p = .033$, 95% CI [0.14, 0.55], and trial 5 ($M = 0.47$, $p = .026$, 95% CI [0.02, 0.59], reflecting reduced SCR amplitude across extinction trials. There was no significant effect of group, $F(2, 42) = 0.33$, $p = .720$, $\eta_p^2 = .02$, or condition, $F(1, 42) = 0.45$, $p = .833$, $\eta_p^2 < .00$. There was no significant interaction for Condition x Group, $F(2, 42) = 1.65$, $p = .204$, $\eta_p^2 = .07$, Trial x Group, $F(6, 129) = 0.35$, $p = .915$, $\eta_p^2 = .02$, Condition x Trial, $F(4, 168) = 1.51$, $p = .201$, $\eta_p^2 = .04$, or Group x Condition x Trial, $F(8, 168) = 0.27$, $p = .974$, $\eta_p^2 = .01$.

Late extinction. A 3 x 2 x 4 (Group [male, EF, ML] x Condition [CS^+, CS^-] x Trial [1, 2, 3, 4, 5]) repeated measures ANOVA revealed a significant main effect for trial, $F(3, 126) = 3.68$, $p = .014$, $\eta_p^2 = .08$. Sidak-corrected pairwise comparisons found the SCR amplitude in trial 1 ($M = 0.71$) was significantly higher than trial 2 ($M = 0.50$), $p = .048$, 95% CI [0.001, 0.44], and trial 5 ($M = 0.44$), $p = .009$, 95% CI [0.05, 0.50], reflecting a general reduction in SCR amplitude across late extinction trials. There were no further significant main effects of group, $F(2, 42) = 0.71$, $p = .498$, $\eta_p^2 = .03$, or condition, $F(1, 42) = 0.01$, $p = .914$, $\eta_p^2 < .00$. There was no significant interaction for Condition x Group, $F(2, 42) = 0.12$, $p = .892$, $\eta_p^2 = .01$, Trial x Group, $F(6, 126) = 1.30$, $p = .261$, $\eta_p^2 = .06$, Condition x Trial, $F(4, 168) =$

1.12, p = .356, η_p^2 = .03, or Group x Condition x Trial, $F(8, 168) = 1.18$, p = .314, η_p^2 = .05.

Fear Extinction Recall

Fear retention index. As described above a fear retention index was calculated**.** A univariate ANOVA was conducted for fear retention index percentage, finding a significant effect for group, $F(2, 42) = 4.27$, p = .021, η_p^2 = .17. As shown in Figure 9, Sidak-corrected pairwise comparisons revealed that the EF group had significantly higher fear retention (M = 57.28%) than the ML group (M = 31.73%), p = .022, 95% CI [2.99, 48.12]. This difference in means suggest that early follicular females have inhibited fear extinction recall. The male group (M = 49.09%) did not significantly differ from either the ML group, p = .142, 95% CI [-4.01, 38.73], or the EF group, p = .752, 95% CI [-30.76, 14.37].

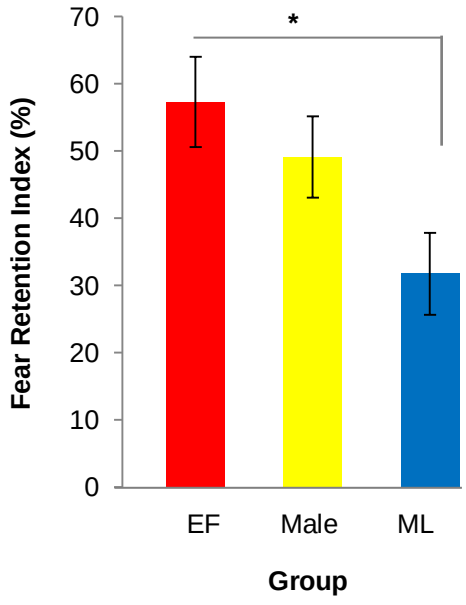

Figure 9. Fear retention index percentage in fear extinction recall for early follicular females, males and mid-luteal females. Error bars are standard error. * $p < .05$

Estradiol and Progesterone

A hierarchical regression was employed to assess estradiol and progesterone as predictors of fear recovery index. As seen in Table 2, regression results for both estradiol and progesterone were non-significant.

Table 2

Hierarchical Multiple Regression Predicting Fear Recovery Index

Predictor	*B* [95%CI]	*t*	*p*	R^2	ΔR^2
Step one				0.001	0.001
Estradiol	0.89 [-10.07, 11.85]	0.16	.871		
Step two				0.03	0.03
Estradiol	4.33 [-8.01, 16.67]	0.71	.483		
Progesterone	-0.06 [-0.15, 0.04]	-1.20	.245		

Note. CI = Confidence interval. $N = 45$

Discussion

The current study aimed to replicate the finding that females in the early follicular phase of the menstrual cycle had diminished fear extinction recall (memory of extinction training from day one), demonstrated by greater SCR amplitude and a high fear retention index compared to males and females in the mid-luteal phase. The results revealed no significant differences in fear extinction recall between the early follicular females and males however, early follicular females had significantly poorer fear extinction recall compared to mid-luteal females suggesting that menstrual phase impacts fear extinction recall in females, thereby replicating previous research. Further, the study aimed to investigate the independent effects of estrogen and progesterone in fear extinction recall across early follicular females,

mid-luteal females and males. The prediction of a negative relationship between estrogen and SCR amplitude on day two was unfound. Unexpectedly, the analysis of independent effects of the two sex hormones did not yield estradiol or progesterone as significant predictors of fear retention.

Fear Acquisition

During fear acquisition all three groups demonstrated robust fear conditioning through a significant escalation in SCR amplitude across the acquisition phase. . This is consistent with previous studies that used SCR data to determine adequate fear acquisition in males, low estrogen, and high estrogen females in healthy and clinical groups (Glover et al., 2012; Milad et al, 2010). Reflecting on reviewed literature by Graham and Milad (2013), Wegerer et al. (2014), and White and Graham (2016) who found consistently higher SCR in the CS^+ condition compared to CS^- condition, the current study also found a significant main effect of condition. This proposes that participants became aware of the CS^+ and electric stimulus pairing, demonstrating contingency awareness (correct identification of the CS^+ stimulus). This was further evidenced by a continuation of elevated SCR in CS^+ trials despite the study only using a 62.5% reinforcement rate. In other words, higher SCR remained persistent even though only five of the eight CS^+ presentations were paired with the electrical stimulus. According to Warren et al. (2014), the elevated SCR in CS^+ presentations demonstrates successful association with danger compared to lower SCR in CS^- presentations which implies an associated with safety signals. Furthermore, data analysis of acquisition SCR did not find a significant effect of group. This parallel's the lack of group effects in studies conducted by Glover et al. (2012), Graham and Milad (2013), and White and Graham (2016). Consequently, learned fear occurred irrespective of sex or menstrual phase and therefore later

significant effects cannot be attributed to differences in fear learning. Lastly, Kelly and Forsyth (2007) argue that increased SCR in fear acquisition demonstrates the paradigms ability to induce learned fear. As an increase in SCR was observed during the acquisition phase this lends support for the current study's adapted fear acquisition and extinction paradigm to effectively induce a conditioned fear response.

Fear Extinction Learning

Fear extinction learning occurred over two phases (early extinction and late extinction). According to Stockhorst & Antov (2016) extinction learning (defined as significantly lower SCR from acquisition) promotes a new form of inhibitory learning concerning the association between CS^+ and CS^-. Participants learn that the aversive stimulus will no longer be administered which consequently reduces the CR. The current study found no main effect of condition, where SCR between CS^+ and CS^- conditions were no longer significant suggesting the conditioning effects seen, in terms of elevated SCR, during acquisition had dissipated. This coincides with results found by Milad et al. (2010), Graham and Milad (2013), and Graham and White (2016) were the effects observed in conditioning were no longer present. In addition, the reduction in SCR in the CS^+ and CS^- condition reflected inhibitory learning (Glover et al., 2013).

The repeated measures ANOVA conducted in early extinction found a significant effect of trial demonstrating a significant decrease in SCR amplitude in the later trials (trial 3 and 5) compared to the early trials (trial 1). This was followed by a further reduction in SCR amplitude in the late extinction trials (trials 1 and 2) with plateau in the later trials (no further significant reductions in SCR after trial 2) suggesting fear extinction learning was successful. The pattern of results obtained for

the extinction learning trials correspond with those of Graham and Milad (2013), Milad et al. (2010), Wegerer et al. (2014), and White and Graham (2016). These studies concluded that significant decreases in SCR across trials demonstrated robust extinction learning. Furthermore, the current study found no main effect of group. Consistent with previous findings by Milad et al. (2010), Wegerer et al. (2014), and White and Graham (2016) the SCR amplitude measured in both early and late extinction in the current study was comparable between the groups. Similar to acquisition, fear extinction learning was comparable across groups with no evidence of differences attributed to sex (males and females) or menstrual phase.

According to Kelly and Forsyth (2007) the US in laboratory based fear acquisition and extinction paradigms is not a true reflection of external ('real world') fear-inducing stimuli. In addition, they argue that the absence of clinical samples (who are known to demonstrate resistant extinction learning, Duits et al., 2015) affords rapid reduction in SCR in experimental sessions. However, in opposition, Miskovic and Keil (2012) argue that while dysfunctional extinction learning (continuous CR to CS^+ and CS^- stimuli without the aversive stimulus) can be present, it is the outcomes that emerge on day two which offers the greatest insight into identifying maladaptive fear extinction recall. As poor memory of extinction learning, despite evidence of adequate in-session extinction, occurs in healthy samples it informs potential reasons behind persistent fear responses observed in clinical samples.

Fear Extinction Recall

The first hypothesis predicted that early follicular females would demonstrate poor fear extinction recall compared to males and mid-luteal females. Most research has compared females in the early and late follicular phases (low and high estrogen,

respectively) and found deficits in extinction recall in early follicular females. This has been attributed to low estradiol levels, but few studies have compared the early follicular phase with the mid-luteal phase, which is characterised by peak progesterone levels and relatively high estradiol levels. The results reported here for the fear extinction recall phase revealed a significant effect of group. Fear extinction recall was significantly lower in early follicular females (demonstrated by high fear retention index) who had higher SCR amplitude during the early and late fear extinction recall trials compared to the mid-luteal females. Furthermore, comparable SCR amplitude between early follicular and mid-luteal females during extinction learning substantiates the view that discrepancies between these groups are a consequence of poor extinction memory consolidation. This partially supports our initial hypothesis. Based on self- reported menstrual phase, this finding suggests that females in episodes of low estrogen tend to experience reduced extinction recall when the original CS^{+} is presented after a 24 hour latency. This finding replicates the increased fear retention found by previous researchers in early follicular females when tested on day two of a fear acquisition and extinction recall paradigm (Graham & Milad; Milad et al., 2010; White & Graham; 2016). However, these findings contradict those of Lonsdorf et al. (2014) who found no difference in SCR data between the follicular and luteal females on day two of their study and Pineles et al. (2016) who discovered mid-luteal females with PTSD demonstrated poor extinction recall in mid-luteal females. The differences found in fear extinction recall in the mid-luteal females in the current study and those of Pineles et al. is likely due to testing healthy versus clinical populations and variations in hormonal synthesis known to occur in individuals with PTSD (Maeng & Milad, 2015; VanElzakker et al., 2014). In contrast to the hypothesis, early follicular females did not differ

significantly from males in fear extinction recall but they displayed a pattern of higher SCR in the early and late extinction recall phase. This trend (increased fear retention in early follicular females) is consistent with previous research by other laboratories who have reported poor fear extinction recall in early follicular females compared to males, nevertheless as this is not a significant finding it should be interpreted with caution. More importantly to note, the overall effect was driven by the differences in fear recovery between the two female groups.

Salivary Measures of Estrogen and Progesterone

Previous research has attributed differences in extinction recall across menstrual phases to low levels of estradiol, and convincing evidence of this has been found in animal studies and in pharmacological-challenge studies in both rats and humans (Graham & Milad, 2011; Graham & Milad, 2013; Milad et al., 2009). In the current study, salivary measures revealed that progesterone clearly discriminated between the menstrual groups. Congruent with expectations, the mid-luteal females displayed significantly greater progesterone levels than the early follicular females and males. As mid-luteal females demonstrated superior fear extinction recall, it is unrealistic to exclude progesterone as a possible mediator in fear extinction recall which parallels findings in animal studies of female rats over the menstrual phase (Milad, Igoe, LeBron-Milad & Novales, 2009). Unexpectedly, in the present study, salivary estradiol did not significantly differ between early follicular and mid-luteal females, although means for early follicular females were in the expected direction (see Figure 7; Lu, Bentley, Gann, Hodges, & Chatterton, 1999). It is possible that this null finding may be due to difficulties associated with the salivary assay for estradiol, as it rapidly deteriorates after thawing (Toone et al., 2013) which in turn can compromise data quality. Thus, this null finding in estradiol could be due to

artefact and requires further exploration before conclusions can be drawn regarding the role of estrogen as an important modulator in achieving adequate fear extinction recall.

The study further aimed to explore how progesterone may impact fear extinction recall. As there is no known research that has directly investigated peak progesterone (in mid-luteal females) in a fear acquisition and extinction recall paradigm, no directional hypothesis was made. A hierarchical regression was conducted in an attempt to evaluate the independent effects of estrogen and progesterone. In the regression analysis detailed in Table 2, neither sex hormone was found to be a significant predictor of fear extinction recall. This finding is surprising, especially for estrogen, as there is a wealth of evidence implicating estrogen as a dominant factor in achieving a reduction in fear retention when testing the adequacy of fear extinction recall in females (Glover et al., 2012; Graham & Milad, 2013; Milad et al., 2010; Stockhorst & Antov, 2016; White & Graham, 2016). However, this null result is likely explained by the difficulties in assaying estradiol from saliva (as described above).

The high level of salivary progesterone appears to be valid, and the regression analysis suggests that there was no significant relationship between progesterone and fear extinction recall. This supports a recent study which found that progesterone was not a significant predictor of fear extinction recall (White & Graham, 2016). Our current findings diverge from Graham and White's study in that they found estrogen to be a significant predictor of fear extinction recall. Possible explanations for the observed differences include that White and Graham used blood samples to test hormonal levels which varies from levels found in saliva. In addition the inconsistency found in the current study may have been a result of potentially

compromised salivary estradiol analysis. In addition, the lack of effects due to progesterone is interesting, considering that progesterone has been found to facilitate extinction recall in animal studies (not included in this study; Milad et al., 2009). Furthermore, combining several factors: emotional properties given to a fearful experience (CS^+ stimuli), high levels of progesterone in the mid-luteal females, a significant main effect of progesterone in fear extinction recall and well-established knowledge that progesterone enhances emotional memory consolidation, one would expect to have found some portion of variance explained (Ertman, Andreano, & Cahill, 2011; Felmingham et al., 2012; LeBron-Milad & Milad, 2012; Maeng & Milad, 2015; Zorawski et al., 2005). Whilst the null effect of progesterone may be a real finding, it does require replication as it may also have been impacted by a lack of statistical power. The required number of participants in the a priori power analysis was not achieved hence this may have led to undetermined effect size and an inflated risk of type II error (Austin & Steyerberg, 2015; Nakagawa, 2004).

Limitations and Future Research

There are limitations within this study worth noting. Firstly, the study was underpowered due to clinical and technical reasons and, most importantly, the difficulty in recruiting females who do not use any form of contraception. Secondly, the limited sample size compromised the detection of adequate effect sizes. Future research should recruit larger samples in order to replicate and extend current and previous research findings.

Another potential limitation relates the collection of salivary samples per participant. The estradiol levels in the mid-luteal females were unexpectedly lower than anticipated. There was a delay in the assay of estradiol as the laboratory's estradiol testing kit supply was depleted. Consequently, estradiol levels were

measured approximately seven days after the progesterone levels. This raises the possibility of inaccurate estradiol measurements. Even though the laboratory reported a one freeze thaw cycle it is unknown how long the saliva samples remained unfrozen and refrigerated or left at room temperature until assay of estradiol. According to Toone et al. (2013) salivary estradiol levels significantly decreases as days pass, irrespective of refrigeration or room temperature, post thawing. An alternative for measuring hormonal status may include blood serum analysis (measuring protein bound estrogens) as it is less sensitive to deterioration (Lu et al., 1999) and may improve the integrity of biological analyses. In addition, even though salivary collection is seen as advantageous in terms of its non-invasiveness and self-collection, salivary hormone levels are known to fluctuate on a daily basis which may confound accurate estradiol readings (Gandara, Leresche, & Mancl, 2007). Consequently, future research considering the use of salivary measures could obtain a saliva sample on both days of the paradigm and average the two reported hormonal levels to improve reliability (Wegerer et al., 2014).

Males have estrogen and progesterone levels comparable to females in the early follicular phase, yet males do not develop anxiety disorders at the same rate of females (Kessler et al., 2005; Milad et al., 2010). This may be influenced by testosterone which is substantially higher in males compared to females (Ackermann et al., 2012). As testosterone is metabolised into estrogen to achieve homeostasis (predominately seen in males when estrogen is low) and a males' capacity to rapidly convert testosterone to estrogen and decrease fear response (Pace-Schott et al., 2013), this makes for interesting analyses of fear extinction recall in males. As the relationship between fear extinction recall and testosterone is rather understudied, future research in the field of sex hormones and fear extinction learning and recall

may include assessment of testosterone to assess its independent effects on extinction recall. Furthermore, as the study only focused exclusively on the effects of estrogen and progesterone it is important to acknowledge the role of cortisol. Cortisol is a hormone known to influence fear learning, fear extinction and memory (de Quervain et al., 2011; Hwang et al., 2013). According to Kirschbaum, Kudielka, Gaab, Schommer and Hellhammer (1999) cortisol levels fluctuate in females over the menstrual cycle and may therefore also influence the disparity in extinction recall found between males and females as well as females in different times of their menstrual cycle. Measuring cortisol in conjunction with other sex hormones can further investigate how the combinations of hormones impact fear acquisition, extinction learning and extinction recall.

Furthermore, with regards to females, estrogen fluctuates across the menstrual cycle. As studies typically only assess fear acquisition, fear extinction and extinction recall in either the early and late follicular phases or the early follicular and luteal phases, the inclusion of a mid-cycle group (where estrogen is at its highest and progesterone remains low) could deepen the understanding of how estrogen and progesterone impact fear extinction recall (Glover et al., 2013; Graham & Milad, 2013; Lonsdorf et al., 2014; Wegerer et al., 2014).

Implications and Conclusions

Bearing in mind that some treatments for anxiety disorders involve fear extinction, it is imperative to incorporate knowledge obtained through empirical studies concerning the role of menstrual phase on fear extinction learning and extinction learning recall in females. Since drawing conclusions based on assessment of hormone levels is inappropriate in this study, clinical implications are derived from self-reported menstrual phase. Targeting exposure related treatment to coincide

with late follicular and luteal phases (when levels of estrogen and progesterone are elevated) may significantly improve treatment outcomes in female clients, such as reduced symptom severity, improved quality of life, and decreased rates of relapse (Duits et al., 2015; Graham & Milad, 2011; Graham & Milad, 2013; White & Graham, 2016).

In summary the current study replicated previous research that have found substantial evidence supporting diminished fear extinction recall in early follicular females. Confirming the initial hypothesis, testing of fear extinction recall in a two-day fear conditioning and extinction paradigm found that early follicular females demonstrated impaired fear extinction recall compared to mid-luteal females. The regression analysis revealed no relationship between progesterone and fear extinction recall and unexpectedly, failed to find a relationship between estrogen and fear extinction recall which may have been due to reduced sensitivity of estradiol salivary data and under-powering of the study. Future research could usefully analyse estrogen and progesterone across the menstrual phase, in order to better understand how progesterone fits into fear extinction learning and recall. Immediately, however, the findings of this study substantiate the view that optimal treatment regimes for anxiety disorders in females requiring fear extinction should include careful consideration of their menstrual cycle.

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Appendix A: Ethics, participant information and consent forms

A1: Ethics approval

A2: Participant information sheet

A3: Participant consent form

Appendix A1

Ethics Amendment Approval: H0012496 Sex differences in fear extinction: The influence of cognitive variables

Katherine Shaw

Mon 11/04/2016 10:51 AM

To: Kim Felmingham <kim.felmingham@utas.edu.au>;

Cc:Cheryl McKay <mckaycl@utas.edu.au>;

Dear Professor Felmingham

Ethics Ref: H0012496
Title: Sex differences in fear extinction: The influence of cognitive variables

This email is to confirm that the following amendment was approved by the Chair of the Tasmania Social Sciences Human Research Ethics Committee on 8/4/2016:

- Change of investigators: removal of Annie To, as she has completed her master's thesis.
- Addition of Honours student Cheryl McKay who will collect an additional experimental group (a midluteal group).
- Revised Information Sheet.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the National Statement on Ethical Conduct in Human Research (NHMRC 2007, updated May 2015).

This email constitutes official approval. If your circumstances require a formal letter of amendment approval, please let us know.
Should you have any queries please do
not hesitate to contact me. Kind
regards
Katherine

Katherine Shaw

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CRICOS 00586B

Appendix A2

Participant Information Sheet

Title: **Sex differences in fear extinction**

Date:

Invitation

You are invited to participate in a research study examining the influence of hormones on fear extinction. This study will be carried out in the Cognitive Neuroscience (ERP) Laboratory at the School of Psychology, University of Tasmania (Hobart). This study is being conducted by Cheryl McKay(Honours student), supervised by Professor Kim Felmingham in partial fulfilment of the requirements of their honours studies in the School of Psychology, University of Tasmania.

What is the purpose of this study?

The purpose of this study is to investigate the influence of hormones on fear conditioning and extinction which are key processes thought to underlie the development and treatment of anxiety disorders. Recent evidence reveals that cognitive variables and sex may influence the rates of fear conditioning and extinction, but few studies have examined the influence of hormones.

Why have I been invited to participate?

You have been invited to participate in this study as you are a healthy male or female, between the ages of 18 to 55, who are not currently taking any medication, and who have no history of psychiatric disorders. We will ask you to complete a questionnaire about these conditions before the experiment begins. In exchange for your participation, you will be learning about the processes of psychological research and you will receive $20 for the hour and a half of experimental research, or 90 minutes of course credit in psychology.

What will I be asked to do?

You will be asked to come in for two testing sessions at the University of Tasmania, the first will take approximately 60 minutes and the second (24 hours later) will take approximately 30 minutes. The study will be run in the Cognitive Neuroscience Laboratory in the School of Psychology. In the first session, you will be asked to sit in a quiet room and complete some questionnaires about your mood, beliefs and cognitive processing style. You will also be asked to fill in a medical history questionnaire, which will ask about the position that you are in your menstrual cycle and contraceptive use (if you are female). The study will also require taking saliva samples (collecting saliva in a small plastic tube). The samples will be

examined by laboratory technicians to measure your current levels of estrogen, progesterone, noradrenaline and cortisol. You will then complete a behavioural task which examines how your body arousal (sweat gland activity) reacts to a mild electrical stimulus that will be administered to your fingertips. You will first be asked to select a level of mild electrical stimulus that feels uncomfortable but not painful to you. This will be done by attaching a finger stimulator to your index finger and delivering the lowest level of electrical stimulus, the level of which will then be increased in small increments until you report that it feels uncomfortable but not painful. You will then be asked to complete the behavioural task. In this task, you will sit in front of a computer screen and small recording disks will be attached to your finger tips to measure your body arousal (via skin conductance). You will then be asked to watch the computer screen on which you will see different coloured circles appear. Following the presentation of some of these coloured circles, you will receive an electrical stimulus which will be set at the level which you have previously chosen. You will also be asked to provide ratings on how much you are expecting to receive the electrical stimulus in the task. The behavioural task will last approximately 15 minutes.

In the second session, you will be asked to provide a second saliva sample and then complete one part of the behavioural task again. This will involve having small recording disks and the finger stimulator to your fingers, and observing the coloured circles. In this second testing session, you may or may not receive electric shocks.

What will happen to my sample after it has been tested?

Your saliva sample will only be used for the purpose of this research study. The saliva samples you provide during the study will be destroyed at the completion of the study. Your saliva samples will not be used for genetic testing or disease markers.

Will I be able to get my sample back if I want?

No, your saliva sample will be destroyed following laboratory analysis.

Will drug or biotechnology companies be able to use my sample for profit in future?

No.

How is this study being paid for?

The study is being sponsored by a grant from the National Health and Medical Research Council.

Are there any possible benefits from participation in this study?

If you decide to participate in this research you will gain experience in research procedures and also some knowledge of underlying mechanisms of anxiety and exposure therapy. If you are enrolled in first year Psychology, you will also receive research participation credit of 1.5 hour for your participation. Furthermore, you will be involved in research that may provide a platform to better understand the mechanisms and processes involved in the extinction of fear, and this may ultimately lead to more efficient and effective exposure treatments for anxiety disorders.

Are there any possible risks from participation in this study?

Prior to commencement of the study you will be asked to sign consent form which will evidence your agreement to participate. You may feel a small amount of arousal or discomfort from the mild electrical stimulus. However, we expect that this arousal or

discomfort to be minimal as the level that is administered will have been selected by you to be uncomfortable but not painful. The technology used to administer this electrical stimulus is very safe and has been used in many previous studies with no adverse effect reported. There will be a researcher with you at all times, and you can discontinue the study at any time without penalty and it will not affect your relationship with the University of Tasmania or the School of Psychology.

What if I change my mind during or after the study?

Participation in this research is entirely voluntary. You may choose to withdraw from the study at any time without prejudice. Deciding to withdraw from this research at any time will not affect your academic standing in any way. You can also choose at this time to withdraw any data previously collected. Participants will be given copies of this information sheet and the statement of informed consent.

What will happen to the information when this study is over?

Your individual data will be treated confidentially, your name will be replaced by an ID number on all data. It will be kept in a locked cabinet or on password secured computers at the School of Psychology at the University of Tasmania for a period of at least five years (with the exception of the medical questionnaires which will be destroyed on completion of the study).

How will the results of the study be published?

Following completion of the research, the data will be published. However, no participant will be personally identifiable in these publications as only group data will be published. A summary of the results of these experiments will be available on the University of Tasmania School of Psychology Web page at www.scieng.utas.edu.au/psychol or will be available by contacting the researchers.

What if I have questions about this study?

The researchers will be available after the testing session to answer any questions you may have. If you have any questions, or would like any additional information regarding this research please contact, Cheryl McKay at mckaycl@utas.edu.au., or Prof Kim Felmingham at Kim.Felmingham@utas.edu.au.

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number H12496.

Thank you for taking the time to consider this study.

This information sheet is for you to keep.

Appendix A3

UTAS

Participant Consent Form

Sex differences in fear extinction: The influence of cognitive variables.

Participant Consent Statement:

1. I agree to take part in the research study named above.
2. I have read and understood the Information Sheet for this study.
3. The nature and possible effects of the study have been explained to me.
4. Any questions that I have asked have been answered to my satisfaction.
5. I understand that the study requires me to attend the Cognitive Neuroscience laboratory at the School of Psychology where my arousal responses will be recorded whilst I view different coloured circles and receive a mild electrical stimulus to my fingers. I understand that I can set the level of this mild electrical stimulus to feel uncomfortable but not painful prior to the task. I understand I will be asked to provide a saliva sample to get estimates of estrogen and progesterone. My involvement in this study will take approximately one hour.
6. I understand that I will be asked about recreational drug habits, use of prescription medication and my menstrual cycle and contraceptive use (if female). I also understand that I should indicate to their experimenter if I have sensitive skin and that I should request a rest if I become fatigued.
7. I understand that all research data will be treated as confidential. I agree that research data gathered for the study may be published provided that I cannot e identified as a participant.
8. I understand that my participation is voluntary and that I may withdraw from participation and/or withdraw my data at any time without prejudice to my academic standing

Participant's name: _____

Participant's signature: _____Date: _____

Investigator Statement

I have explained this research and the implications of participation in it to this volunteer and I believe that the consent is informed and that she understands the implications of participation

Investigator's name: _____

Investigator's signature: _____Date: _____

Appendix B: Questionnaires

B1: Medical checklist

B2: The Depression Anxiety Stress Scale (DASS 21)

B3: The Difficulty Emotion Regulation Scale (DERS)

B4: The Catastrophic Cognitions Questionnaire – Modified (CCQ-M)

B5: The Belief about Emotions Scale (BAES)

Appendix B1

NAME: _____ DATE: _____ PARTICIPANT ID: _____

GROUP: EF ML MALE CONSENT OBTAINED: _____

AGE: _____ GENDER_____MEDICATION:

_____

DATE OF MENSTRUATION: _____ DAY OF CYCLE: _____

SALIVA SAMPLE COLLECTED: _____

TIME SINCE WAKING: _____ SMOKER: _____ PER DAY: _____

WEIGHT: _____ HEIGHT: _____ BMI: _____

DASS (ANX): _____ DASS (DEP): _____ DASS (STR):_____

CCQ: _____ STIMULUS: _____mA

DERS: _____

BAES: _____

CS^{+}: _____ IDENTIFIED: _____

ADDITIONAL INFORMATION:

Appendix B2

DASS21

Name: *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (eg, in the hands)	0	1	2	3
8	I felt that I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about anything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

DASS Severity Ratings

The DASS is a **quantitative** measure of distress along the axes of depression, anxiety (symptoms of psychological arousal) and stress (the more cognitive, subjective symptoms of anxiety). It is **not** a categorical measure of clinical diagnoses.

Emotional syndromes like depression and anxiety are intrinsically dimensional – they vary along a continuum of severity (independent of the specific diagnosis). Hence the selection of a single cut-off for a specific diagnosis can be correctly recognised as experiencing considerable symptoms and as being at high risk of further problems.

However for clinical purposes it can be helpful to have 'labels' to characterise degree of severity relative to the population. Thus the following cut-off scores have been developed for defining mild/moderate/severe/extremely severe scores for each DASS scale.

Note: the severity labels are used to describe the full range of scores in the population, so 'mild' for example means that the person is above the population mean but probably still way below the typical severity of someone seeking help (ie it does not mean a mild level of disorder).

The individual DASS scores do not define appropriate interventions. They should be used in conjuction with all clinical information available to you in determining appropriate treatment for any individual.

With the above information in mind, we offer the following guidelines based on full (42 item) scores (if using the DASS 21 item version, multiply the score obtained by 2).

DASS Severity Ratings
(if using the DASS 21 item version, multiply the score obtained by 2)

	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely Severe	28+	20+	34

Source: Psychology Department, UNSW - www.psy.unsw.edu.au/dass

DASS Scoring Template

Best printed on an overhead transparency sheet

	S
	A
	D
	A
	D
	S
	A
	S
	A
	D
	S
	S
	D
	S
	A
	D
	D
	S
	A
	A
	D

Apply template to sheet and sum scores for each scale.
For short (21-item) version, multiply sum by 2.

Appendix B3

Participant ID: Date: Assessment #:

Difficulties in Emotion Regulation Scale (DERS)

Please indicate how often the following statements apply to you by writing the appropriate number from the scale below on the line beside each item.

1	2	3	4	5
almost never (0-10%)	sometimes (11-35%)	about half the time (36-65%)	most of the time (66-90%)	almost always (91-100%)

_____ 1) I am clear about my feelings.

_____ 2) I pay attention to how I feel.

_____ 3) I experience my emotions as overwhelming and out of control.

_____ 4) I have no idea how I am feeling.

_____ 5) I have difficulty making sense out of my feelings.

_____ 6) I am attentive to my feelings.

_____ 7) I know exactly how I am feeling.

_____ 8) I care about what I am feeling.

_____ 9) I am confused about how I feel.

_____ 10) When I'm upset, I acknowledge my emotions.

_____ 11) When I'm upset, I become angry with myself for feeling that way.

_____ 12) When I'm upset, I become embarrassed for feeling that way.

_____ 13) When I'm upset, I have difficulty getting work done.

_____ 14) When I'm upset, I become out of control.

_____ 15) When I'm upset, I believe that I will remain that way for a long time.

_____ 16) When I'm upset, I believe that I will end up feeling very depressed.

_____ 17) When I'm upset, I believe that my feelings are valid and important.

_____ 18) When I'm upset, I have difficulty focusing on other things.

_____ 19) When I'm upset, I feel out of control.

_____ 20) When I'm upset, I can still get things done.

Participant ID: Date: Assessment #:

1	2	3	4	5
almost never (0-10%)	sometimes (11-35%)	about half the time (36-65%)	most of the time (66-90%)	almost always (91-100%)

_____ 22) When I'm upset, I know that I can find a way to eventually feel better.

_____ 23) When I'm upset, I feel like I am weak.

_____ 24) When I'm upset, I feel like I can remain in control of my behaviors.

_____ 25) When I'm upset, I feel guilty for feeling that way.

_____ 26) When I'm upset, I have difficulty concentrating.

_____ 27) When I'm upset, I have difficulty controlling my behaviors.

_____ 28) When I'm upset, I believe there is nothing I can do to make myself feel better.

_____ 29) When I'm upset, I become irritated at myself for feeling that way.

_____ 30) When I'm upset, I start to feel very bad about myself.

_____ 31) When I'm upset, I believe that wallowing in it is all I can do.

_____ 32) When I'm upset, I lose control over my behavior.

_____ 33) When I'm upset, I have difficulty thinking about anything else.

_____ 34) When I'm upset I take time to figure out what I'm really feeling.

_____ 35) When I'm upset, it takes me a long time to feel better.

_____ 36) When I'm upset, my emotions feel overwhelming.

SUBSCALE SCORING:**

1. Nonacceptance of emotional responses (NONACCEPT): 11, 12, 21, 23, 25, 29
2. Difficulty engaging in Goal-directed behavior (GOALS): 13, 18, 20R, 26, 33
3. Impulse control difficulties (IMPULSE): 3, 14, 19, 24R, 27, 32
4. Lack of emotional awareness (AWARENESS): 2R, 6R, 8R, 10R, 17R, 34R
5. Limited access to emotion regulation strategies (STRATEGIES): 15, 16, 22R, 28, 30, 31, 35, 36
6. Lack of emotional clarity (CLARITY): 1R, 4, 5, 7R, 9

Total score: sum of all subscales

**"R" indicates reverse scored item

REFERENCE:

Gratz, K. L. & Roemer, L. (2004). Multidimensional assessment of emotion regulation and dysregulation: Development, factor structure, and initial validation of the Difficulties in Emotion Regulation Scale. *Journal of Psychopathology and Behavioral Assessment, 26,* 41-54.

Appendix B4

Name _____ Date _____

AGE _____ SEX M _____ F _____

Instructions: The questionnaire aims at measuring your beliefs and thoughts regarding the following items. Sometimes these items are believed to be DANGEROUS. Please read the items carefully, and choose a number from the scale given below to rate the extent you believe them to be dangerous to you. Write the number you chose in the box opposite each item. For example by writing 1, you believe that the item is NOT AT ALL dangerous. By writing 5, you believe that the item is EXTREMELY DANGEROUS. Do not spend too much time, and try to answer all of them.

1	2	3	4	5
Not at all Dangerous	A little Dangerous	Quite Dangerous	Very Dangerous	Extremely Dangerous

1. Feeling edgy ..... :___:
2. Having an accident ..... :___:
3. Mind not functioning normally ..... :___:
4. Being miserable ..... :___:
5. Being injured ..... :___:
6. Unable to think rationally ..... :___:
7. Feeling shaky ..... :___:
8. Having a stroke ..... :___:
9. Unable to control thinking ..... :___:
10. Being agitated ..... :___:
11. Being ill ..... :___:
12. Losing memory ..... :___:
13. Unable to relax ..... :___:
14. Being suffocated ..... :___:
15. Being mentally blocked ..... :___:
16. Being alarmed ..... :___:
17. Being attacked ..... :___:
18. Being out of senses ..... :___:
19. Being angry ..... :___:
20. Losing sight ..... :___:
21. Being mentally blurred ..... :___:

Fig. 1. Catasptophic Cognitions Questionnaire - Modified

Appendix B5

Beliefs about Emotions Scale

Rimes, K.A. & Chalder, T. (2010). The Beliefs about Emotions Scale: Validity, Reliability and Sensitivity to Clinical Change. *Journal of Psychosomatic Research, 68,* 295-282.

The version given overleaf does not have a time referent, but you may wish to ask participants to rate their beliefs over the past week, if you are interested in using it before and treatment, for example.

Response options are provided from left to right: "Totally agree", "Agree very much", "Agree slightly", "Neutral", "Disagree slightly", "Disagree very much" and "Totally disagree". Responses are scored 6, 5, 4, 3, 2, 1, and 0 respectively.

For further information please contact Katharine Rimes at Katharine.Rimes@kcl.ac.uk

Please see overleaf for the scale

Beliefs about Emotions Scale (Rimes & Chalder, 2010)

Please tick the column that best describes how you think. Please note that because people are different, there are no right or wrong answers to these statements. To decide whether a given answer is typical of your way of looking at things, simply keep in mind how you think most of the time.

	Totally agree	Agree very much	Agree slightly	Neutral	Disagree slightly	Disagree very much	Totally disagree
It is a sign of weakness if I have miserable thoughts.							
If I have difficulties I should not admit them to others.							
If I lose control of my emotions in front of others, they will think less of me.							
I should be able to control my emotions.							
If I am having difficulties it is important to put on a brave face.							
If I show signs of weakness then others will reject me.							
I should not let myself give in to negative feelings.							
I should be able to cope with difficulties on my own without turning to others for support.							
To be acceptable to others, I must keep any difficulties or negative feelings to myself.							
It is stupid to have miserable thoughts.							
It would be a sign of weakness to show my emotions in public.							
Others expect me to always be in control of my emotions.							

Appendix C

Salivary data analysis

Appendix C

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Sample Preparation and Analysis

Kim Felmingham Aug 2016

estradiol and progesterone

Sample collection and storage.

Samples were stored frozen at -20°C until assay. All samples underwent one freeze thaw cycle.

Sample preparation

On the day of assay appropriate number of samples were thawed analysed using commercially available kits (Salimetrics, USA) according to the manufacturers instructions. Thawed samples were centrifuged at 1500 x *g* for 15 min to collect clear saliva and this saliva was used without further processing for all assays. All samples were brought to room temperature before adding to assay wells and all samples were analysed in duplicate.

Salivary Progesterone

Introduction

The Salimetrics progesterone assay kit is a competitive immunoassay specifically designed to measure salivary cortisol. It uses a specific polyclonal antibody to competitively bind endogenous salivary progesterone (sP) and a specified concentration of added horseradish peroxidase labeled progesterone (hrpP). The degree of competition between endogenous and added progesterone can be calculated to measure salivary progesterone. The reaction can be summed as follows:

$$Ab + sP + hrpP \Rightarrow Ab/sP + Ab/hrpP$$

By measuring the concentration of Ab/hrpP we can measure the amount of salivary progesterone (sP) present in the subject saliva.

Test Principle

A microtitre plate is coated with polyclonal antibodies to progesterone. Progesterone in unknown saliva samples competes with progesterone linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound progesterone peroxidase (Ab/hrpP) is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of progesterone peroxidase detected is inversely proportional to the amount of salivary progesterone present.

For the purpose of publications and thesis it is sufficient to state that salivary progesterone was measured using a commercially available ELISA assay (Salimetrics, USA) according to the manufacturers instructions.

Assay Performance

Salivary progesterone correlates well with matched serum progesterone concentrations; r = 0.80 (females), r = 0.87 (males).

Assay sensitivity = 5.0 pg/mL.

Intra assay variability (within assay) 5.4%

Inter assay variability (between assays) 5.9%

Salivary 17β-ESTRADIOL

Introduction

The Salimetrics™ estradiol kit is a competitive immunoassay specifically

designed and validated for the quantitative measurement of salivary estradiol.

It uses a specific polyclonal antibody to competitively bind endogenous salivary estradiol (sE) and a specified concentration of added horseradish peroxidase labeled progesterone (hrpE). The degree of competition between endogenous and added estradiol can be calculated to measure salivary estradiol. The reaction can be summed as follows:

$$Ab + sE + hrpE \Rightarrow Ab/sE + Ab/hrpE$$

By measuring the concentration of Ab/hrpE we can measure the amount of salivary estradiol (sE) present in the subject saliva.

Test Principle

A microtitre plate is coated with rabbit antibodies to estradiol. Estradiol in

standards and unknowns competes with estradiol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound estradiol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with 2-molar sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of estradiol peroxidase detected is inversely proportional to the amount of estradiol present.

For the purpose of publications and thesis it is sufficient to state that salivary estradiol was measured using a commercially available ELISA assay (Salimetrics, USA) according to the manufacturers instructions.

Assay Performance

Salivary estradiol correlates well with matched serum estradiol concentrations; r = 0.80 (females).

Assay sensitivity = 0.1 pg/mL.

Intra assay variability (within assay) 5.9%

Inter assay variability (between assays) 6.4%

Observations

All samples ran well no observations.

Mark Longster.

Managing Director

Appendix D: SPSS Output

One-way ANOVA: DASS Depression

Tests of Between-Subjects Effects

Dependent Variable: DASS (Depression)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	15.201[a]	2	7.600	.449	.641	.021	.898	.118
Intercept	611.346	1	611.346	36.108	.000	.462	36.108	1.000
GROUP	15.201	2	7.600	.449	.641	.021	.898	.118
Error	711.111	42	16.931					
Total	1361.000	45						
Corrected Total	726.311	44						

Estimates

Dependent Variable: DASS (Depression)

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	2.923	1.141	.620	5.226
MALE	3.812	1.029	1.737	5.888
ML	4.375	1.029	2.299	6.451

One-way ANOVA: DASS Anxiety

Tests of Between-Subjects Effects

Dependent Variable: DASS (Anxiety)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	9.078[a]	2	4.539	.164	.849	.008	.328	.074
Intercept	869.452	1	869.452	31.440	.000	.428	31.440	1.000
GROUP	9.078	2	4.539	.164	.849	.008	.328	.074
Error	1161.500	42	27.655					
Total	2033.000	45						
Corrected Total	1170.578	44						

Estimates

Dependent Variable: DASS (Anxiety)

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	5.000	1.459	2.057	7.943
MALE	3.875	1.315	1.222	6.528
ML	4.375	1.315	1.722	7.028

One-way ANOVA: DASS Stress

Tests of Between-Subjects Effects

Dependent Variable: DASS (Stress)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	58.313[a]	2	29.157	.890	.418	.041	1.780	.193
Intercept	2273.907	1	2273.907	69.394	.000	.623	69.394	1.000
GROUP	58.313	2	29.157	.890	.418	.041	1.780	.193
Error	1376.264	42	32.768					
Total	3753.000	45						
Corrected Total	1434.578	44						

Estimates

Dependent Variable: DASS (Stress)

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	6.615	1.588	3.411	9.819
MALE	6.125	1.431	3.237	9.013
ML	8.688	1.431	5.799	11.576

One-way ANOVA: Catastrophic Cognitions Questionnaire-Modified

Tests of Between-Subjects Effects

Dependent Variable: Catastrophic Cognitions Questionnaire-M

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	944.859[a]	2	472.430	2.405	.103	.103	4.811	.459
Intercept	141670.375	1	141670.375	721.279	.000	.945	721.279	1.000
GROUP	944.859	2	472.430	2.405	.103	.103	4.811	.459
Error	8249.452	42	196.416					
Total	151549.000	45						
Corrected Total	9194.311	44						

Estimates

Dependent Variable: Catastrophic Cognitions Questionnaire-M

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	58.385	3.887	50.540	66.229
MALE	50.188	3.504	43.117	57.258
ML	60.563	3.504	53.492	67.633

One-way ANOVA: The Difficulty Emotion Regulation Scale

Tests of Between-Subjects Effects

Dependent Variable: Diffficulty in Emotion Regulation Scale

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	1767.183[a]	2	883.591	2.791	.073	.117	5.582	.520
Intercept	304679.816	1	304679.816	962.427	.000	.958	962.427	1.000
GROUP	1767.183	2	883.591	2.791	.073	.117	5.582	.520
Error	13296.129	42	316.574					
Total	318710.250	45						
Corrected Total	15063.311	44						

Estimates

Dependent Variable: Diffficulty in Emotion Regulation Scale

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	90.692	4.935	80.734	100.651
MALE	82.344	4.448	73.367	91.320
ML	75.000	4.448	66.023	83.977

One-way ANOVA: Age

Tests of Between-Subjects Effects

Dependent Variable: Age of participant

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	42.262[a]	2	21.131	.498	.611	.023	.996	.126
Intercept	26890.674	1	26890.674	633.454	.000	.938	633.454	1.000
GROUP	42.262	2	21.131	.498	.611	.023	.996	.126
Error	1782.938	42	42.451					
Total	28910.000	45						
Corrected Total	1825.200	44						

Estimates

Dependent Variable: Age of participant

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	25.000	1.807	21.353	28.647
MALE	25.437	1.629	22.150	28.725
ML	23.250	1.629	19.963	26.537

One-way ANOVA: Body mass index (BMI)

Tests of Between-Subjects Effects

Dependent Variable: Body Mass Index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	25.091[a]	2	12.546	.846	.436	.039	1.693	.186
Intercept	22797.600	1	22797.600	1538.168	.000	.973	1538.168	1.000
GROUP	25.091	2	12.546	.846	.436	.039	1.693	.186
Error	622.493	42	14.821					
Total	23812.940	45						
Corrected Total	647.584	44						

Estimates

Dependent Variable: Body Mass Index

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	21.523	1.068	19.368	23.678
MALE	23.250	.962	21.308	25.192
ML	23.075	.962	21.133	25.017

One-way ANOVA: Hours awake

Tests of Between-Subjects Effects

Dependent Variable: Hours since waking

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	19.068[a]	2	9.534	1.189	.314	.054	2.379	.246
Intercept	1483.929	1	1483.929	185.118	.000	.815	185.118	1.000
GROUP	19.068	2	9.534	1.189	.314	.054	2.379	.246
Error	336.677	42	8.016					
Total	1823.500	45						
Corrected Total	355.744	44						

Estimates

Dependent Variable: Hours since waking

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	6.654	.785	5.069	8.239
MALE	5.625	.708	4.197	7.053
ML	5.031	.708	3.603	6.460

One-way ANOVA: Stimulus (mA)

Tests of Between-Subjects Effects

Dependent Variable: STIMULUS mA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	.632[a]	2	.316	1.758	.185	.077	3.517	.348
Intercept	178.257	1	178.257	991.358	.000	.959	991.358	1.000
GROUP	.632	2	.316	1.758	.185	.077	3.517	.348
Error	7.552	42	.180					
Total	190.190	45						
Corrected Total	8.184	44						

Estimates

Dependent Variable: STIMULUS mA

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	1.831	.118	1.593	2.068
MALE	2.119	.106	1.905	2.333
ML	2.050	.106	1.836	2.264

One-way ANOVA: Progesterone

Tests of Between-Subjects Effects

Dependent Variable: Progesterone levels pg/ml

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	160626.310[a]	2	80313.155	14.764	.000	.413	29.528	.998
Intercept	271582.226	1	271582.226	49.925	.000	.543	49.925	1.000
GROUP	160626.310	2	80313.155	14.764	.000	.413	29.528	.998
Error	228471.457	42	5439.797					
Total	682150.133	45						
Corrected Total	389097.767	44						

Estimates

Dependent Variable: Progesterone levels pg/ml

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	38.462	20.456	-2.819	79.744
MALE	34.608	18.439	-2.603	71.819
ML	161.106	18.439	123.895	198.317

Pairwise Comparisons

Dependent Variable: Progesterone levels pg/ml

(I) Male, Early Follicular (EF) Mid-Luteal (ML)	(J) Male, Early Follicular (EF) Mid-Luteal (ML)	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
EF	MALE	3.854	27.540	.999	-64.629	72.337
	ML	-122.644*	27.540	.000	-191.127	-54.161
MALE	EF	-3.854	27.540	.999	-72.337	64.629
	ML	-126.498*	26.076	.000	-191.342	-61.654
ML	EF	122.644*	27.540	.000	54.161	191.127
	MALE	126.498*	26.076	.000	61.654	191.342

One-way ANOVA: Estradiol

Tests of Between-Subjects Effects

Dependent Variable: Estroidal levels pg/ml

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	3.388[a]	2	1.694	3.540	.038	.144	7.080	.627
Intercept	214.903	1	214.903	449.145	.000	.914	449.145	1.000
GROUP	3.388	2	1.694	3.540	.038	.144	7.080	.627
Error	20.096	42	.478					
Total	242.164	45						
Corrected Total	23.484	44						

Estimates

Dependent Variable: Estroidal levels pg/ml

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	2.066	.192	1.679	2.453
MALE	1.952	.173	1.604	2.301
ML	2.569	.173	2.220	2.918

One-way ANOVA: Baseline SCL

Tests of Between-Subjects Effects

Dependent Variable: Baseline SCL

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	3.159[a]	2	1.580	.079	.924	.004	.157	.061
Intercept	2494.953	1	2494.953	124.233	.000	.747	124.233	1.000
GROUP	3.159	2	1.580	.079	.924	.004	.157	.061
Error	843.481	42	20.083					
Total	3383.105	45						
Corrected Total	846.640	44						

Estimates

Dependent Variable: Baseline SCL

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	7.092	1.243	4.584	9.600
MALE	7.670	1.120	5.409	9.931
ML	7.683	1.120	5.422	9.944

RM ANOVA: Habituation

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Intercept	136.963	1	136.963	159.150	.000	.791	159.150	1.000
GROUP	.509	2	.254	.295	.746	.014	.591	.094
Error	36.145	42	.861					

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Condition	Sphericity Assumed	.017	1	.017	.098	.756	.002	.098	.061
	Greenhouse-Geisser	.017	1.000	.017	.098	.756	.002	.098	.061
	Huynh-Feldt	.017	1.000	.017	.098	.756	.002	.098	.061

	Lower-bound	.017	1.000	.017	.098	.756	.002	.098	.061
Condition * GROUP	Sphericity Assumed	.576	2	.288	1.675	.200	.074	3.350	.333
	Greenhouse-Geisser	.576	2.000	.288	1.675	.200	.074	3.350	.333
	Huynh-Feldt	.576	2.000	.288	1.675	.200	.074	3.350	.333
	Lower-bound	.576	2.000	.288	1.675	.200	.074	3.350	.333
Error(Condition)	Sphericity Assumed	7.221	42	.172					
	Greenhouse-Geisser	7.221	42.000	.172					
	Huynh-Feldt	7.221	42.000	.172					
	Lower-bound	7.221	42.000	.172					
Trial	Sphericity Assumed	5.442	3	1.814	9.899	.000	.191	29.698	.998
	Greenhouse-Geisser	5.442	2.752	1.977	9.899	.000	.191	27.247	.996
	Huynh-Feldt	5.442	3.000	1.814	9.899	.000	.191	29.698	.998
	Lower-bound	5.442	1.000	5.442	9.899	.003	.191	9.899	.867
Trial * GROUP	Sphericity Assumed	.887	6	.148	.807	.567	.037	4.840	.310
	Greenhouse-Geisser	.887	5.505	.161	.807	.557	.037	4.440	.295
	Huynh-Feldt	.887	6.000	.148	.807	.567	.037	4.840	.310
	Lower-bound	.887	2.000	.443	.807	.453	.037	1.613	.179

Error(Trial)	Sphericity Assumed	23.088	126	.183					
	Greenhouse-Geisser	23.088	115.601	.200					
	Huynh-Feldt	23.088	126.000	.183					
	Lower-bound	23.088	42.000	.550					
Condition * Trial	Sphericity Assumed	.182	3	.061	.314	.815	.007	.941	.109
	Greenhouse-Geisser	.182	2.745	.066	.314	.798	.007	.861	.107
	Huynh-Feldt	.182	3.000	.061	.314	.815	.007	.941	.109
	Lower-bound	.182	1.000	.182	.314	.578	.007	.314	.085
Condition * Trial * GROUP	Sphericity Assumed	.830	6	.138	.716	.637	.033	4.296	.276
	Greenhouse-Geisser	.830	5.489	.151	.716	.625	.033	3.930	.263
	Huynh-Feldt	.830	6.000	.138	.716	.637	.033	4.296	.276
	Lower-bound	.830	2.000	.415	.716	.495	.033	1.432	.163
Error(Condition*Trial)	Sphericity Assumed	24.344	126	.193					
	Greenhouse-Geisser	24.344	115.271	.211					
	Huynh-Feldt	24.344	126.000	.193					
	Lower-bound	24.344	42.000	.580					

Estimates

Measure: MEASURE_1

Trial	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	.762	.060	.641	.883
2	.719	.061	.596	.841
3	.468	.063	.341	.595
4	.531	.068	.394	.668

Pairwise Comparisons

Measure: MEASURE_1

(I) Trial	(J) Trial	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.043	.059	.978	-.121	.207
	3	.294*	.063	.000	.120	.469
	4	.231*	.069	.011	.040	.423
2	1	-.043	.059	.978	-.207	.121
	3	.251*	.069	.004	.061	.441

	4	.188*	.067	.045	.003	.373
3	1	-.294*	.063	.000	-.469	-.120
	2	-.251*	.069	.004	-.441	-.061
	4	-.063	.056	.840	-.217	.091
4	1	-.231*	.069	.011	-.423	-.040
	2	-.188*	.067	.045	-.373	-.003
	3	.063	.056	.840	-.091	.217

RM ANOVA: Acquisition

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Condition	1.000	.000	0	.	1.000	1.000	1.000
Trial	.965	1.439	5	.920	.976	1.000	.333
Condition * Trial	.916	3.587	5	.610	.947	1.000	.333

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Intercept	184.841	1	184.841	160.493	.000	.793	160.493	1.000
GROUP	.004	2	.002	.002	.998	.000	.004	.050
Error	48.371	42	1.152					

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Condition	Sphericity Assumed	3.168	1	3.168	9.145	.004	.179	9.145	.840
	Greenhouse-Geisser	3.168	1.000	3.168	9.145	.004	.179	9.145	.840
	Huynh-Feldt	3.168	1.000	3.168	9.145	.004	.179	9.145	.840
	Lower-bound	3.168	1.000	3.168	9.145	.004	.179	9.145	.840
Condition * GROUP	Sphericity Assumed	.851	2	.425	1.228	.303	.055	2.456	.253
	Greenhouse-Geisser	.851	2.000	.425	1.228	.303	.055	2.456	.253
	Huynh-Feldt	.851	2.000	.425	1.228	.303	.055	2.456	.253
	Lower-bound	.851	2.000	.425	1.228	.303	.055	2.456	.253
Error(Condition)	Sphericity Assumed	14.549	42	.346					
	Greenhouse-Geisser	14.549	42.000	.346					
	Huynh-Feldt	14.549	42.000	.346					
	Lower-bound	14.549	42.000	.346					
Trial	Sphericity Assumed	.806	3	.269	.871	.458	.020	2.612	.236

	Greenhouse-Geisser	.806	2.929	.275	.871	.456	.020	2.550	.233
	Huynh-Feldt	.806	3.000	.269	.871	.458	.020	2.612	.236
	Lower-bound	.806	1.000	.806	.871	.356	.020	.871	.149
Trial * GROUP	Sphericity Assumed	.869	6	.145	.469	.830	.022	2.815	.186
	Greenhouse-Geisser	.869	5.857	.148	.469	.826	.022	2.748	.184
	Huynh-Feldt	.869	6.000	.145	.469	.830	.022	2.815	.186
	Lower-bound	.869	2.000	.434	.469	.629	.022	.938	.122
Error(Trial)	Sphericity Assumed	38.879	126	.309					
	Greenhouse-Geisser	38.879	123.004	.316					
	Huynh-Feldt	38.879	126.000	.309					
	Lower-bound	38.879	42.000	.926					
Condition * Trial	Sphericity Assumed	.727	3	.242	1.150	.332	.027	3.451	.304
	Greenhouse-Geisser	.727	2.841	.256	1.150	.331	.027	3.268	.295
	Huynh-Feldt	.727	3.000	.242	1.150	.332	.027	3.451	.304
	Lower-bound	.727	1.000	.727	1.150	.290	.027	1.150	.182
Condition * Trial * GROUP	Sphericity Assumed	1.214	6	.202	.961	.455	.044	5.766	.369
	Greenhouse-Geisser	1.214	5.682	.214	.961	.452	.044	5.460	.357

	Huynh-Feldt	1.214	6.000	.202	.961	.455	.044	5.766	.369
	Lower-bound	1.214	2.000	.607	.961	.391	.044	1.922	.206
Error(Condition*Trial)	Sphericity Assumed	26.538	126	.211					
	Greenhouse-Geisser	26.538	119.329	.222					
	Huynh-Feldt	26.538	126.000	.211					
	Lower-bound	26.538	42.000	.632					

Estimates

Measure: MEASURE_1

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	.814	.072	.668	.960
2	.626	.056	.512	.739

Pairwise Comparisons

Measure: MEASURE_1

(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.189*	.062	.004	.063	.314
2	1	-.189*	.062	.004	-.314	-.063

RM ANOVA: Early Extinction

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Condition	1.000	.000	0	.	1.000	1.000	1.000
Trial	.571	22.638	9	.007	.771	.878	.250
Condition * Trial	.742	12.086	9	.209	.874	1.000	.250

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Intercept	151.433	1	151.433	106.873	.000	.718	106.873	1.000
GROUP	.938	2	.469	.331	.720	.016	.662	.099
Error	59.512	42	1.417					

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Condition	Sphericity Assumed	.009	1	.009	.045	.833	.001	.045	.055
	Greenhouse-Geisser	.009	1.000	.009	.045	.833	.001	.045	.055
	Huynh-Feldt	.009	1.000	.009	.045	.833	.001	.045	.055
	Lower-bound	.009	1.000	.009	.045	.833	.001	.045	.055
Condition * GROUP	Sphericity Assumed	.629	2	.315	1.650	.204	.073	3.301	.329
	Greenhouse-Geisser	.629	2.000	.315	1.650	.204	.073	3.301	.329
	Huynh-Feldt	.629	2.000	.315	1.650	.204	.073	3.301	.329
	Lower-bound	.629	2.000	.315	1.650	.204	.073	3.301	.329
Error(Condition)	Sphericity Assumed	8.008	42	.191					
	Greenhouse-Geisser	8.008	42.000	.191					
	Huynh-Feldt	8.008	42.000	.191					
	Lower-bound	8.008	42.000	.191					
Trial	Sphericity Assumed	5.702	4	1.425	5.506	.000	.116	22.025	.974

	Greenhouse-Geisser	5.702	3.083	1.849	5.506	.001	.116	16.975	.939
	Huynh-Feldt	5.702	3.513	1.623	5.506	.001	.116	19.346	.959
	Lower-bound	5.702	1.000	5.702	5.506	.024	.116	5.506	.630
Trial * GROUP	Sphericity Assumed	.718	8	.090	.347	.946	.016	2.772	.163
	Greenhouse-Geisser	.718	6.166	.116	.347	.915	.016	2.137	.147
	Huynh-Feldt	.718	7.027	.102	.347	.932	.016	2.435	.155
	Lower-bound	.718	2.000	.359	.347	.709	.016	.693	.102
Error(Trial)	Sphericity Assumed	43.493	168	.259					
	Greenhouse-Geisser	43.493	129.485	.336					
	Huynh-Feldt	43.493	147.566	.295					
	Lower-bound	43.493	42.000	1.036					
Condition * Trial	Sphericity Assumed	1.472	4	.368	1.510	.201	.035	6.040	.461
	Greenhouse-Geisser	1.472	3.495	.421	1.510	.208	.035	5.278	.427
	Huynh-Feldt	1.472	4.000	.368	1.510	.201	.035	6.040	.461
	Lower-bound	1.472	1.000	1.472	1.510	.226	.035	1.510	.225
Condition * Trial * GROUP	Sphericity Assumed	.534	8	.067	.274	.974	.013	2.193	.136
	Greenhouse-Geisser	.534	6.990	.076	.274	.963	.013	1.916	.129

	Huynh-Feldt	.534	8.000	.067	.274	.974	.013	2.193	.136
	Lower-bound	.534	2.000	.267	.274	.762	.013	.548	.090
Error(Condition*Trial)	Sphericity Assumed	40.928	168	.244					
	Greenhouse-Geisser	40.928	146.784	.279					
	Huynh-Feldt	40.928	168.000	.244					
	Lower-bound	40.928	42.000	.974					

Estimates

Measure: MEASURE_1

Trial	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	.778	.078	.621	.935
2	.640	.083	.472	.808
3	.498	.069	.357	.638
4	.527	.070	.385	.669
5	.472	.069	.333	.611

Pairwise Comparisons

Measure: MEASURE_1

(I) Trial	(J) Trial	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.138	.083	.669	-.108	.384
	3	.280*	.090	.033	.014	.547
	4	.251	.092	.088	-.020	.521
	5	.306*	.096	.026	.023	.589
2	1	-.138	.083	.669	-.384	.108
	3	.142	.065	.287	-.049	.334
	4	.113	.060	.494	-.064	.289
	5	.168	.078	.315	-.063	.399
3	1	-.280*	.090	.033	-.547	-.014
	2	-.142	.065	.287	-.334	.049
	4	-.030	.055	1.000	-.191	.132
	5	.026	.066	1.000	-.169	.221
4	1	-.251	.092	.088	-.521	.020
	2	-.113	.060	.494	-.289	.064

	3	.030	.055	1.000	-.132	.191
	5	.055	.066	.994	-.138	.249
5	1	-.306*	.096	.026	-.589	-.023
	2	-.168	.078	.315	-.399	.063
	3	-.026	.066	1.000	-.221	.169
	4	-.055	.066	.994	-.249	.138

RM ANOVA: Late Extinction

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Condition	1.000	.000	0	.	1.000	1.000	1.000
Trial	.565	23.069	9	.006	.751	.853	.250
Condition * Trial	.666	16.455	9	.058	.840	.966	.250

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Intercept	134.083	1	134.083	96.121	.000	.696	96.121	1.000
GROUP	1.977	2	.989	.709	.498	.033	1.417	.162
Error	58.587	42	1.395					

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Condition	Sphericity Assumed	.004	1	.004	.012	.914	.000	.012	.051
	Greenhouse-Geisser	.004	1.000	.004	.012	.914	.000	.012	.051
	Huynh-Feldt	.004	1.000	.004	.012	.914	.000	.012	.051
	Lower-bound	.004	1.000	.004	.012	.914	.000	.012	.051
Condition * GROUP	Sphericity Assumed	.080	2	.040	.115	.892	.005	.230	.066
	Greenhouse-Geisser	.080	2.000	.040	.115	.892	.005	.230	.066
	Huynh-Feldt	.080	2.000	.040	.115	.892	.005	.230	.066
	Lower-bound	.080	2.000	.040	.115	.892	.005	.230	.066
Error(Condition)	Sphericity Assumed	14.660	42	.349					

	Greenhouse-Geisser	14.660	42.000	.349					
	Huynh-Feldt	14.660	42.000	.349					
	Lower-bound	14.660	42.000	.349					
Trial	Sphericity Assumed	3.843	4	.961	3.679	.007	.081	14.717	.874
	Greenhouse-Geisser	3.843	3.002	1.280	3.679	.014	.081	11.045	.792
	Huynh-Feldt	3.843	3.413	1.126	3.679	.010	.081	12.556	.831
	Lower-bound	3.843	1.000	3.843	3.679	.062	.081	3.679	.466
Trial * GROUP	Sphericity Assumed	2.722	8	.340	1.303	.245	.058	10.425	.586
	Greenhouse-Geisser	2.722	6.004	.453	1.303	.261	.058	7.824	.496
	Huynh-Feldt	2.722	6.826	.399	1.303	.254	.058	8.894	.535
	Lower-bound	2.722	2.000	1.361	1.303	.282	.058	2.606	.267
Error(Trial)	Sphericity Assumed	43.871	168	.261					

	Greenhouse-Geisser	43.871	126.085	.348					
	Huynh-Feldt	43.871	143.336	.306					
	Lower-bound	43.871	42.000	1.045					
Condition * Trial	Sphericity Assumed	1.095	4	.274	1.106	.356	.026	4.424	.343
	Greenhouse-Geisser	1.095	3.361	.326	1.106	.352	.026	3.717	.311
	Huynh-Feldt	1.095	3.863	.283	1.106	.355	.026	4.272	.336
	Lower-bound	1.095	1.000	1.095	1.106	.299	.026	1.106	.177
Condition * Trial * GROUP	Sphericity Assumed	2.334	8	.292	1.179	.314	.053	9.434	.534
	Greenhouse-Geisser	2.334	6.722	.347	1.179	.319	.053	7.927	.482
	Huynh-Feldt	2.334	7.726	.302	1.179	.315	.053	9.110	.524
	Lower-bound	2.334	2.000	1.167	1.179	.317	.053	2.358	.244
Error(Condition*Trial)	Sphericity Assumed	41.567	168	.247					

	Greenhouse-Geisser	41.567	141.172	.294						
	Huynh-Feldt	41.567	162.240	.256						
	Lower-bound	41.567	42.000	.990						

Estimates

Measure: MEASURE_1

Trial	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	.712	.065	.580	.844
2	.492	.063	.365	.618
3	.568	.082	.403	.732
4	.536	.092	.349	.723
5	.435	.063	.308	.562

Pairwise Comparisons

Measure: MEASURE_1

(I) Trial	(J) Trial	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.220*	.074	.048	.001	.438
	3	.144	.101	.831	-.155	.443
	4	.175	.095	.528	-.106	.457
	5	.276*	.077	.009	.049	.504
2	1	-.220*	.074	.048	-.438	-.001
	3	-.076	.073	.975	-.293	.141
	4	-.044	.075	1.000	-.266	.177
	5	.056	.057	.981	-.112	.225

3	1	-.144	.101	.831	-.443	.155
	2	.076	.073	.975	-.141	.293
	4	.031	.069	1.000	-.171	.234
	5	.132	.070	.490	-.074	.338
4	1	-.175	.095	.528	-.457	.106
	2	.044	.075	1.000	-.177	.266
	3	-.031	.069	1.000	-.234	.171
	5	.101	.064	.724	-.087	.289
5	1	-.276*	.077	.009	-.504	-.049
	2	-.056	.057	.981	-.225	.112
	3	-.132	.070	.490	-.338	.074
	4	-.101	.064	.724	-.289	.087

One-way ANOVA: Fear retention index

Tests of Between-Subjects Effects

Dependent Variable: % Fear Recovery Index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	5042.727[a]	2	2521.364	4.269	.021	.169	8.539	.714
Intercept	94437.107	1	94437.107	159.910	.000	.792	159.910	1.000
GROUP	5042.727	2	2521.364	4.269	.021	.169	8.539	.714
Error	24803.639	42	590.563					
Total	122110.073	45						
Corrected Total	29846.367	44						

Estimates

Dependent Variable: % Fear Recovery Index

Male, Early Follicular (EF) Mid-Luteal (ML)	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EF	57.279	6.740	43.677	70.881
MALE	49.085	6.075	36.825	61.346
ML	31.726	6.075	19.466	43.987

Pairwise Comparisons

Dependent Variable: % Fear Recovery Index

(I) Male, Early Follicular (EF) Mid-Luteal (ML)	(J) Male, Early Follicular (EF) Mid-Luteal (ML)	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
EF	MALE	8.194	9.074	.752	-14.371	30.758
	ML	25.553*	9.074	.022	2.989	48.117
MALE	EF	-8.194	9.074	.752	-30.758	14.371
	ML	17.359	8.592	.142	-4.006	38.725
ML	EF	-25.553*	9.074	.022	-48.117	-2.989
	MALE	-17.359	8.592	.142	-38.725	4.006

Regression Analysis

Model Summary[c]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.025[a]	.001	-.023	26.33767	.001	.027	1	43	.871
2	.184[b]	.034	-.012	26.20073	.033	1.451	1	42	.235

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	43.327	12.608		3.436	.001	17.900	68.753		
	Estroidal levels pg/ml	.886	5.435	.025	.163	.871	-10.074	11.847	1.000	1.000
2	(Constant)	40.358	12.782		3.157	.003	14.562	66.153		
	Estroidal levels pg/ml	4.328	6.115	.121	.708	.483	-8.013	16.669	.782	1.279
	Progesterone levels pg/ml	-.057	.048	-.207	-1.204	.235	-.153	.039	.782	1.279

a. Dependent Variable: % Fear Recovery Index